Docket No. : 532552000101
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:	Confirmation No.: 6691
Paul TARDI, *et al.*	Group Art Unit: 1616
Serial No.: 10/417,631	Examiner: Ali Soroush
Filing Date: April 16, 2003	
For: COMPOSITIONS FOR DELIVERY OF DRUG COMBINATIONS	

DECLARATION OF LAWRENCE D. MAYER
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Lawrence D. Mayer, declare as follows:

1. I am President and Head of Research of Celator Pharmaceuticals, Inc., the assignee herein. I have been actively engaged in drug delivery research, both academically and in industrial settings for over 22 years. A copy of my *curriculum vitae* is attached as Exhibit A.

2. I agree with the statement by the Examiner in his Office Action dated November 15, 2007, "...the state of the art is very high in terms of formulating the liposomal sustained release compositions..." Indeed there were numerous references in the art at the time

of the present application describing a wide range of approaches and methods for controlling the loading as well as release of liposomal agents with the latter being widely examined for a number of antineoplastic agents. Methods of modulating drug retention and release in multiple delivery vehicles, including liposomes and polymer-based nanoparticles, are well known to those of skill in the art. This literature includes an extensive compendium of data on actual release rates for a multiplicity of drugs from a multiplicity of particulate carriers.

3. Examples of methods for controlling liposomal drug release include:

a) the use of a transmembrane ion gradients, particularly pH gradients generated with, for example, citrate, ammonium sulfate or an ionophore as well as using various degrees of said pH gradients;

b) the use of transmembrane osmotic gradients of varying degrees;

c) modulating the class (*e.g.*, anionic, neutral, pegylated), acyl chain length and/or amount of individual phospholipids used in a mixture;

d) the addition of cholesterol;

e) altering the drug-to-lipid ratio;

f) selecting delivery vehicles with specific phase transition temperatures

4. Below is a list of references for each of the above categories. It should be noted that his list is only meant to provide representative methods and numerous other examples and related approaches were readily available:

a) Ionic Gradients

i. Cullis, P.R., *et al.*, Influence of pH gradients on the transbilayer transport of drugs, lipids, peptides and metal ions into large unilamellar vesicles. *Biochim. Biophys. Acta* (1997) 1331:187-211.

ii. Burke, T., and Gao, X., Stabilization of topotecan in low pH liposomes composed of distearoylphosphatidylcholine. *J. Pharm. Sciences* (1994) July; 83(7):967-969.

iii. Bowman, N., *et al.*, Liposomal vincristine which exhibits increased drug retention and increased circulation longevity cures mice bearing P388 tumors. *Cancer Res.* (1994) June 1; 54:2830:2833.

iv. Haran, G., *et al.*, Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. Biochim. *Biophys. Acta* (1993) Sept 19; 1151(2):201-215.

v. Fenske, D.B., *et al.*, Ionophore-mediated uptake of ciprofloxacin and vincristine into large unilamellar vesicles exhibiting transmembrane ion gradients. Biochim. *Biophys. Acta* (1998) Nov 11; 1414(1-2):188-204.

vi. Bowman, N., *et al.*, Optimization of the retention properties of vincristine in liposomal systems. *Biochim. Biophys. Acta* (1993) 1152:253-258.

vii. Clerc, S., and Barenholz, Y., Loading of amphipathic weak acids into liposomes in response to transmembrane calcium acetate gradients. *Biochim. Biophys. Acta* (1995) Dec 13; 1240(2):257-265.

viii. Redelmeier, T., *et al.*, Proton flux in large unilamellar vesicles in response to membrane potentials and pH gradients. *Biophys. J.* (1989) Aug; 56(2):385-393.

ix. US Patent 5,077,056 (Published December 31, 1991) – see Example 1 Part A – "Active Loading Using Na+ /K+ Gradients"

b) Osmotic Gradients

i. Mui, B.L., *et al.*, Influence of plasma on the osmotic sensitivity of large unilamellar vesicles prepared by extrusion. *J. Biol. Chem.* (1994) Mar 11;269(10).

ii. Allen, T.M., and Cleland, L.G., Serum-induced leakage of liposome contents. *Biochim. Biophys. Acta* (1980) 597:418-426.

c) Lipid Composition

i. Horowitz, A.T., *et al.*, *In vitro* cytotoxicity of liposome-encapsulated doxorubicin: dependence on liposome composition and drug release. *Biochim. Biophys. Acta* (1992) 1109:203-209.

ii. Frezard, F., and Garnier-Sullerot, A., Permeability of lipid bilayer to anthracycline derivatives. Role of the bilayer composition and of the temperature. *Biochim. Biophys. Acta* (1998) 1389:13-22.

iii. Lim, H.J., *et al.*, Role of drug release and liposome-mediated drug delivery in governing the therapeutic activity of liposomal mitoxantrone used to treat human A431 and LS180 solid tumors. *J. Pharmacol. Exp. Ther.* (2000) Jan;-292(1):337-345.

iv. Forssen, E.A., and Tokes, Z.A., Improved therapeutic benefits of doxorubicin by entrapment in anionic liposomes. *Cancer Res.* (1983) Feb; 43:546-550.

d) Cholesterol Content

i. Dos Santos, N., *et al.,* Improved retention of Idarubicin after intravenous injection obtained for cholesterol-free liposomes. *Biochim. Biophys. Acta* (2002) Jan; 1561:188-201.

ii. Ogihara-Umeda, I., and Kojima, S., Cholesterol enhances the delivery of liposome-encapsulated gallium-67 to tumors. *Eur. J. Nucl. Med.* (1989) 15:612-617.

iii. Fielding, R.M., and Abra, R.M., Factors affecting the release rate of terbutaline from liposome formulations after intratracheal instillation in the guinea pig. *Pharm. Res.* (1992) Feb; 9(2):220-223.

e) **Drug-to-Lipid Ratio**

i. Mayer, L., *et al.*, Influence of vesicle size, lipid composition, and drug-to-lipid ratio on the biological activity of liposomal doxorubicin in mice. *Cancer Res.* (1989) Nov. 1; 49:5922-5930.

f) **Phase Transition Temperature**

i. Hays, L.M., *et al.*, Factors affecting leakage of trapped solutes from phospholipid vesicles during thermotropic phase transitions. *Cryobiology* (2001) Mar; 42(2):88-102.

ii. Hayashi, H., *et al.*, Temperature-controlled release property of phospholipid vesicles bearing a thermo-sensitive polymer. *Biochim. Biophys. Acta* (1996) 1280:127-134.

iii. Anyarambhatla, G., and Needham, D., Enhancement of the phase transitions permeability of DPPC liposomes by incorporation of MPPC: A new temperature-sensitive liposome for use with mild hyperthermia. *J. Liposome Res.* (1999) 9(4):491-506.

iv. Kong, G., *et al.*, Efficacy of liposomes and hyperthermia in a human tumor xenograft model: Importance of triggered drug release. *Cancer Res.* (2000) Dec. 15; 60:6950-6957.

5. In addition, a similar database existed at the time of the invention describing methods for controlling drug release from natural and synthetic non-liposomal delivery vehicles including micelles, nanoparticles, microspheres and drug-polymer conjugates. Below is a list of references which detail various methods for controlling the release of therapeutic agents from non-liposomal delivery vehicles:

a) Genta, I., *et al.*, Different molecular weight chitosan microspheres: influence on drug loading and release. *Drug Dev. Ind. Pharm.* (1998) Aug; 24(8):779-784.

b) Kim, H., and Fassihi, R., Application of binary polymer system in drug release rate modulation. 2. Influence of formulation variables and hydrodynamic conditions on release kinetics. *J. Pharm. Sci.* (1997) Mar; 86(3):323-328.

c) Kawaguchi, T., *et al.*, Control of drug release with a combination of prodrug and polymer matrix: Antitumor activity and release profiles of 2',3'-Diacyl-5-fluoro-2'-deoxyuridine from Poly(3-hydroxybutyrate) microspheres. *J. Pharm. Sci.* (1992) June; 81(6):508-512.

d) Anderson, B.C., *et al.*, Understanding drug release from poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) gels. *J. Control Release* (2001) Jan 29; 70(1-2):157-167.

e) Muller, R.H., *et al.*, Solid lipid nanoparticles (SLN) for controlled drug delivery – A review of the state of the art. *Eur. J. Pharm. Biopharm.* (2000) Jul; 50(1):161-177.

6. Not only does the art describe various release rates for various drugs in various combinations, the above-cited documents also describe factors that can be controlled in predictable ways to accelerate or decelerate the release of any particular drug, such as a lipophilic drug, a hydrophilic drug, a charged drug or a neutral drug. For example, the rate of release of a lipophilic drug can be decelerated by employing phospholipids with longer acyl chains and can be controlled by adjusting the pH of the internal solution.

7. While the Examiner acknowledges that there is significant skill in the state of the prior art for controlling drug release of single drugs from drug delivery vehicles, he asserts in the November 15, 2007 Office Action that the state of the prior art is not high in terms of "...releasing the two neoplastic agents in the same non-antagonistic ratios." Actually the amount of knowledge of this subject was high at the time of the invention, but experiments were not

reported in the prior art until the present invention, since the knowledge that *ratios* are important AND must be maintained *in vivo* was never appreciated in the art. Therefore the known methods of controlling drug release were never applied to coordinating the release of drug combinations even though practitioners knew how to do it. Once the desirability of controlling ratios was revealed as a result of the present invention, those of skill in the art are able to use the abundant references available to construct delivery vehicles with matched release kinetics of two active agents thereby controlling their ratio after *in vivo* administration.

8. For drugs with dissimilar characteristics in which a single delivery vehicle coordinating release of the two drugs is not ideal as described in the specification (paragraphs 0023-0024, 0115, 0117 and 0130), two different carriers modulating the release kinetics of each drug individually can be designed to coordinate the ratio in the blood as demonstrated in Examples 8, 12, and 15 in the specification. The two drug-containing delivery vehicles can be mixed and co-administered *in vivo*. Thus, for example, a combination of a negatively charged drug with a positively charged one might be handled in this way. Coordinated release both from co-encapsulated drugs and drugs formulated separately is illustrated in Examples 8-9, 12-13 and 15-16.

9. Given the breadth of methods available in the prior art for controlling drug release, there are multiple features of each delivery vehicle which could be readily designed to achieve the coordinated release of two encapsulated agents whether co-encapsulated or encapsulated in separate delivery vehicles such that a desired synergistic ratio is maintained in the blood. This could be achieved without undue experimentation based on the extensive data-

base on actual drug release rates from drug delivery vehicles available in the literature for a wide range of drug classes and delivery vehicles.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at Vancouver, CANADA, on January 14, 2008

Lawrence D. Mayer

Curriculum Vitae

DR. LAWRENCE D. MAYER

EXHIBIT A

EDUCATION

Ph.D. Biochemistry (1983) University of Minnesota, Dr. Gary L. Nelsestuen, Supervisor
Thesis: Protein-Membrane Interactions Involved in the Prothrombinase System

B.Sc. Majors in both Chemistry and Biology (1978, *Summa Cum Laude*) Wartburg College

PROFESSIONAL EMPLOYMENT

2003-Present **Founder, President and Head of Research, Celator Pharmaceuticals**

1999-2002 **Founder, President and Chief Executive Officer, Celator Pharmaceuticals**

2004-Present **Adjunct Scientist, BC Cancer Agency**

1992-Present **Adjunct Professor, Faculty of Pharmaceutical Sciences, University of B.C.**

1992-2003 **Senior Research Scientist, Division of Medical Oncology, B.C. Cancer Agency**
Research programs in the development of liposomal drug delivery systems, multidrug resistance and novel chemotherapeutic agents. Graduated 7 Ph.D. students, 3 M.Sc. students and supervised 16 postdoctoral fellows. Awarded over $4M in research funding.

1999-2001 **Research Supervisor, Experimental Medicine, University of B.C.**

1992-2002 **Director, Investigational Drug Program, B.C. Cancer Agency**
Established and directored translational research and development activities of the Investigational Drug Program including GMP production of clinical supplies in the BCCA clean room facility and GLP testing. Co-ordinated proposals for preclinical and clinical testing of new anticancer agents for outside companies as well as development programs for agents generated within the Agency.

1990-1992 **Program Director, QLT Inc.**
Project manager for topical photosensitizer development program. Responsible for implementation of Quality Management system to ensure compliance with HPB and FDA regulatory requirements. Also responsible for directing formulation efforts of topical photosensitizers, coordinating activities between various departments (toxicology, pharmacology, clinical, *etc.*), preparing budget estimates and project administration. Member of Development Management Group responsible for establishing short term and long term corporate drug development strategy.

1987-1990 **Scientific Director, The Canadian Liposome Co. Ltd.**
Project leader of lead liposomal anticancer agent (TLC D-99) at CLC and its parent company The Liposome Co., Inc. (Princeton, N.J.). Responsible for coordinating R&D activities with current time tables for ongoing clinical trials and anticipated production requirements for future trials. Participated in preparing IND submissions to US FDA and Canadian HPB for clinical testing of TLC D-99. Co-directed research projects concerning the mechanism of action of liposomal anticancer pharmaceuticals. Responsible for developing budget requirements for active research projects.

1986-1987 **Research Associate, Biochemistry Dept., University of B.C.**

1983-1986 **Canadian MRC Postdoctoral Fellow, Biochemistry Department, University of B.C.**

AREAS OF SPECIAL INTEREST AND ACCOMPLISHMENTS

- Spearheaded the translation of Celator's CombiPlex fixed-drug ratio combination chemotherapy technology from the lab into the clinic, resulting in two products being evaluated in clinical trials.
- Part of Celator Senior Management team responsible for securing $10MCDN Series A and $40MUS Series B Venture Capital Financings
- Established new paradigm for developing drug combinations for cancer chemotherapy where synergistic drug:drug ratios are fixed inside liposomes for delivery to tumors. This allows drug combinations to be developed based on maximum efficacy rather than on tolerability.
- Co-founder of Celator Technologies as a spin-off of BCCA laboratory focussed on delivery of drug combinations
- Identified novel applications for and characterized the molecular and therapeutic activities of Bcl-2 antisense oligonucleotides used to chemosensitize human solid tumor xenografts which were implemented by Genta, the company developing Genesense.
- Established novel liposome delivery platform based on reactive, cholesterol-free membranes for enhanced disease site-selective activity.
- Identified enhanced lung cancer antitumor activity of vinca alkaloid derivative anhydrovinblastine and led translational research activities that resulted in IND submission for Phase I clinical testing.
- Discovered and characterized use of liposomal anticancer drug formulations in combination with P-glycoprotein inhibitors to effectively treat multidrug resistant tumors, an approach that was validated in later clinical trials.
- Led discovery and translational research activities to develop an optimized liposomal vincristine formulation and led preparation of the IND submission.
- Played leading role in basic and applied research for development of liposomal doxorubicin formulation currently approved for market use in Europe.
- Established and directed operations of the Investigational Drug Program at the BC Cancer Agency. This Program has been granted an Establishment License by the Canadian TPP as a GMP manufacturing clean room and testing facility for clinical trial supplies. This is the only academic center in Canada to hold such accreditation.

HONOURS AND AWARDS

- Nomination in the Ernst & Young Entrepreneur of the Year awards program, in recognition of determination and inspiration in building a thriving business – and a strong and vibrant country (2003)
- Canadian Medical Research Council Postdoctoral Fellow (1983-1986)
- Dissertation Fellowship, University of Minnesota (1982)
- Wayne Page Memorial Outstanding Student-Athlete Award, Wartburg College (1977)

TEACHING

Areas of special interest and accomplishments

- Worked on course coordination committee to develop Cancer Biology graduate course (Path 500)
- Active yearly participation in training of undergraduate students through summer research programs with Faculty of Pharmaceutical Sciences, Pharmacology Dept. and Biochemistry Dept. at UBC

Courses Taught at UBC

Session	Course Number	Scheduled Hours	Class Size	Hours Taught			
				Lectures	Tutorials	Labs	Other
1996-1997	MedGen 421		150	4			
1996-1997	Path 531		17	4			
1997-1998	MedGen 421		150	4			
1997-1998	Path 531		13	4			
1997-1998	Path 548		7	3			
1998-1999	MedGen 421		150	4			
1999	Phar 414				40		
1999-2000	Phar 510		12	8			
2001	Phar 550		50	4			
2001	Phar 414				30		

Graduate students supervised: 7

Students co-supervised: 3

Member of supervisory committees: 12

Postdoctoral fellows supervised: 17

SUMMARY OF GRANTS & CONTRACTS

Total # of grants/contracts held: 29
Range of years: 1992 -- 2006
Total amount of grant money: $9,345,248

INVITED PRESENTATIONS

- "Association of prothrombin, Factor X and Factor V with phospholipid monolayers" FASEB meetings, Chicago, Illinois, May 1982.
- "Protein-membrane interactions involved in the prothrombinase system" Biomembranes Discussion Group, University of British Columbia, December 1982.

- "Influence of transmembrane ion gradients on the transbilayer distribution of dibucaine" Pharmacology Seminar Series, University of British Columbia, October 1985.
- "Optimization of the therapeutic activity of liposomal doxorubicin" FASEB Liposomal Drug Delivery Conference, Saxton, Vermont, July 1987.
- "Liposomal anticancer formulations with improved therapeutic potential" Oncology Experimental Therapeutics Seminar Series, Roswell Park Memorial Institute, Buffalo, New York, March 1990.
- "Development of liposomal vincristine preparations with improved therapeutic potential" Advanced Therapeutics Seminar Series, British Columbia Cancer Agency, Vancouver, B.C., October 1990.
- "Liposomes as intravenous drug delivery systems" Canadian Society of Hospital Pharmacists Annual General Meeting, Vancouver, B.C., November 1990.
- "Pharmacodynamics of liposomal anticancer agents" University of British Columbia, Faculty of Pharmaceutical Sciences Invited Lecture Series, Vancouver, B.C., March 1994.
- "Formulation, toxicity and pharmacology of liposomal doxorubicin" Liposome Research Days Conference, Vancouver, B.C., June 1994.
- "Pharmacology of vincristine encapsulated in sphingomyelin-cholesterol liposomes" AACR Annual General Meeting Discussion Session, Toronto, Ontario, March 1995.
- "Cellular pharmacology of verapamil in P388-ADR cells in vitro" AACR Annual General Meeting Discussion Session, Toronto, Ontario, March 1995.
- "Preclinical and clinical studies with liposomal vincristine" Third Drug Delivery Symposium, Shizuoka, Japan, June 1995.
- "Membrane Properties Control the Therapeutic Activity of Liposomal Vincristine" Fourth Liposomal Research Conference, Freiburg, Germany, August 1995.
- "The Use of Liposomes to Target Anticancer Agents to Solid Tumors" Gordon Research Conference on Drug Carriers in Biology and Medicine, Ventura, CA, February 1996.
- "Strategies for Optimizing Liposomal Anticancer Agents" PharmTech Conference, East Rutherford, N.J., September 1996.
- "The Use of Liposomes in Therapeutic and Mechanistic Studies of Multidrug Resistance" Canadian Multidrug Resistance Roundtable Meeting, Toronto, Ont., March 1997.
- "Current Trends in Cancer Chemotherapy" Bridging the Strait of Georgia Cancer Research Symposium, Sidney, B.C., May 1997.
- "Therapeutic and Mechanistic Studies on the Pharmacology of Multidrug Resistance" Univ. of British Columbia Dept. of Surgery Seminar Series, Vancouver, B.C., July 1997.
- "In Vivo Entrapment of Doxorubicin Utilising pH Gradient Liposomes" Gordon Research Conference on Drug Carriers in Biology and Medicine, Ventura, CA, February 1998.
- Strategic Development of Biopharmaceuticals" Pictet Biotechnology Seminar, Vancouver, B.C., September 1998.
- "Designing Liposomal Anticancer Agents to Overcome MDR in Combination with the P-glycoprotein Inhibitor PSC 833" International Conference of Anticancer Research, Thessaloniki, Greece, October, 1998.
- "Optimization of Liposomal Anticancer Drug Formulations for Specific Therapeutic Applications" University of London Liposomes in Biomedical Applications Symposium, London, UK, Dec. 1999.
- "Therapeutic and Pharmacokinetic Properties of Doxorubicin combined with Bcl-2 Antisense Oligonucleotide Treatment" Poster Discussion Session, AACR Annual Meeting, San Francisco, CA, April 2000.
- "Matching Drug Release Kinetics with Therapeutic Applications for Liposomal Anticancer Drug Formulations" International Liposome Research Society Conference, Napa, CA, April 2000.
- "Characterization of a Novel Thermosensitive Liposomal Doxorubicin Formulation for Tumor Specific Drug Exposure" Congress of International Society of Hyperthermia Oncology, Taegu, Korea, April 2000.

- "Application of Liposomal Drug Delivery Systems for Treating Multidrug Resistant Tumors" Pharmacology 2001, Vancouver, BC, March 2001.

CONFERENCE PARTICIPATION (ORGANIZER, KEYNOTE SPEAKER, ETC.)

- Session Chair, "Drug Delivery and Tumor Vascular Targeting" Amer. Assoc. Cancer Res. General Meeting, Philadelphia, PA, April 13, 1999.
- Session Chair, "Gene Targeting" Bridging the Strait of Georgia Cancer Symposium, Vancouver Island, 1998,
- Organizer, Bridging the Strait of Georgia Cancer Symposium, Vancouver Island, 1996 and 1998.
- Session Chair, "Developments in Antisense Technologies", Gordon Research Conference on Drug Carriers in Biology and Medicine, Ventura, CA, February, 1996.
- Session Chair, "Steric Stabilisation", Fourth Liposomal Research Conference, Freiburg, Germany, August 1995.

MEMBERSHIPS ON SCHOLARLY SOCIETIES, INCLUDING OFFICES HELD AND DATES

- 2005-present: Editorial Academy of the *International Journal of Oncology* (invited)
- 2003-present: American Association for the Advancement of Science
- 1997-present: International Liposome Society
- 1994-present: Parenteral Drug Association
- 1992-present: American Association of Cancer Research
- 1992-present: American Association of Pharmaceutical Sciences
- 1994-present: B.C. Biotechnology Alliance
- 1989-1993: Stanley Park Zoological Society (Elected member of Board of Directors)
- 1992-1994: Biopharmaceutical Innovation Resource Centre/Economic Regional Diversification Agreement Steering Committee

EDITORSHIPS

- 2001-Present: Journal of Experimental Therapeutics and Oncology
- 2001-Present: Molecular Cancer Therapeutics
- 1999-present: International Journal of Oncology

JOURNAL REVIEWER

- 2007-Present European Journal of Pharmaceutical Biopharmaceutics
- 2001-Present Molecular Cancer Therapeutics
- 2001-Present Journal of Pharmaceutical Research
- 1999-Present International Journal of Oncology
- 1998-Present Journal of Pharmaceutical Sciences
- 1998-Present: Cytometry
- 1997-Present Clinical Cancer Research
- 1996-Present: Molecular Pharmacology

- 1996-Present: European Journal of Pharmaceutical Science
- 1996-Present: Journal of Pharmacology and Experimental Therapeutics
- 1995-Present: International Journal of Cancer
- 1994-Present: European Journal of Cancer
- 1993-Present Journal of Liposome Research
- 1992-Present: Journal of the American Chemical Society
- 1991-Present: Cancer Chemotherapy and Pharmacology
- 1991-Present: British Journal of Cancer
- 1990-Present: Journal of Biological Chemistry
- 1990-Present: Cancer Research
- 1987-Present: Biochemistry
- 1987-Present: Biochimica et Biophysica Acta

GRANTING AGENCY REVIEWER

- 2000-2004: Medical Research Council of Canada, Pharmaceutical Sciences Panel
- 1999-2004: Canadian Breast Cancer Research Foundation
- 1997, 1998, 2000: Alberta Cancer Board (External Reviewer)
- 1995-2001: National Cancer Institute of Canada, Panel G, Pharmacology,
- 1995, 1997, 1998: Medical Research Council of Canada (Pharmaceutics Panel, Cancer B Panel)
- 1995-1996: National Cancer Institute of Canada, Panel J

CONSULTANT (ORGANIZATION AND DATES)

- Photovision Pharmaceuticals, Inc., Jenkintwon, PA 2000-2001
- Duke University Hyperthermia Program (M. Dewhirst, Chair), Durham, N.C. 1998-2002
- Elan Pharmaceuticals, Inc., Ireland 1998-1999
- IGT Pharma, Inc., Vancouver, B.C. 1997-2001
- Angiogenesis Technologies, Vancouver B.C. 1995-1997
- QLT Phototherapeutics, Vancouver, B.C. 1993-2000
- Inex Pharmaceuticals, Corp., Burnaby, B.C. 1993-1999

EXTERNAL EXAMINER

- University of Sydney (Pharmaceutical Sciences) June, 2000
- Simon Fraser University (Biology Department) July, 1998

OTHER SERVICE TO THE COMMUNITY

Guest Science Lectures at Mulgrave School, 1997, 1998, 2000
Mulgrave School (North Vancouver) Planning Committee, 1996-1997

PUBLICATIONS

Journals/Refereed

1. **Mayer LD** and Nelsestuen GL (1981) "Calcium and Prothrombin-Induced Lateral Phase Separation in Membranes" Biochemistry 20, 2457-2463.

2. Pusey ML, **Mayer LD**, Wei GJ, Bloomfield VA and Nelsestuen GL (1982) "Kinetic and Hydrodynamic Analysis of Blood Clotting Factor V-Membrane Binding" Biochemistry 21, 5262-5269.

3. **Mayer LD** and Nelsestuen GL (1983) "Membrane Lateral Phase Separation Induced by Proteins of the Prothrombinase Complex" Biochim Biophys Acta. 734, 48-53.

4. **Mayer LD**, Nelsestuen GL and Brockman HL (1983) "Prothrombin Association with Phospholipid Monolayers" Biochemistry, 22, 316-321.

5. **Mayer LD**, Pusey ML, Griep MA and Nelsestuen GL (1983) "Association of Blood Coagulation Factors V and X with Phospholipid Monolayers" Biochemistry, 22, 6266-6233.

6. Nayar R, **Mayer LD**, Hope MJ and Cullis PR (1984) "Phosphatidic Acid as a Calcium Ionophore in Large Unilamellar Vesicle Systems" Biochim Biophys Acta. 777, 343-346.

7. **Mayer LD**, Bally MB, Hope MJ and Cullis PR (1985) "Uptake of Dibucaine into Large Unilamellar Vesicles in Response to a Membrane Potential" J. Biol. Chem. 260, 802-808.

8. **Mayer LD**, Bally MB, Hope MJ and Cullis PR (1985) "Uptake of Antineoplastic Agents into Large Unilamellar Vesicles in Response to a Membrane Potential" Biochim Biophys Acta. 816, 294-302.

9. **Mayer LD**, Hope MJ, Cullis PR and Janoff AS (1985) "Solute Distributions and Trapping Efficiencies Observed in Freeze-thawed Multilamellar Vesicles" Biochim Biophys Acta, 817:193-196.

10. Richards RL, Habbersett RC, Scher I, Janoff AS, Schieren HP, **Mayer LD**, Cullis PR and Alving CR (1986) "Influence of Vesicle Size on Complement-Dependent Immune Damage to Liposomes" Biochim Biophys Acta. 855:223-230.

11. **Mayer LD**, Bally MB and Cullis PR (1986) "Uptake of Adriamycin into Large Unilamellar Vesicles in Response to a pH Gradient" Biochim Biophys Acta. 857:123-126.

12. **Mayer LD**, Hope MJ and Cullis PR (1986) "Vesicles of Variable Sizes Produced by a Rapid Extrusion Procedure" Biochim Biophys Acta 858:161-168.

13. **Mayer LD**, Bally MB, Hope MJ and Cullis PR (1986) "Techniques for Encapsu-lating Bioactive Agents into Liposomes" Chem. Phys. Lipids 40, 333-345.

14. Hope MJ, Bally MB, **Mayer LD**, Janoff AS and Cullis PR (1986) "Generation of Multilamellar and Unilamellar Phospholipid Vesicles" Chem. Phys. Lipids 40, 89-107.

15. Wong KF, Parmar YI, **Mayer LD**, Pritchard PH and Cullis PR (1987) "Detection of Protein-free Lipoprotein Analogues with an Apolar Lipid Core by Freeze-etch Electron Microscopy" Biochim Biophys Acta. 921:411-414.

16. Brenner DE, Arakali AV, **Mayer LD**, Ginsberg RS and Kanter P (1988) "Comparison of Liposomal Doxorubicin and Free Doxorubicin Pharmacokinetics in Rabbit" Clin. Pharmacol. Therapeut. 43, 125.

17. **Mayer LD**, Wong KF, Menon K, Chong C, Harrigan PR and Cullis PR (1988) "Influence of Ion Gradients on the Transbilayer Distribution of Dibucaine in Large Unilamellar Vesicles" Biochemistry 27, 2053-2060.

18. Bally MB, **Mayer LD**, Loughrey H, Redelmeier T, Madden TD, Wong K, Harrigan PR, Hope MJ and Cullis PR (1988) "Dopamine Accumulation in Large Unilamellar Vesicle Systems Induced by Transmembrane Ion Gradients" Chem. Phys. Lipids 47, 97-107.

19. Janoff AS, Kurtz CL, Jablonski RL, Minchey SR, Boni LT, Gruner SM, Cullis PR, **Mayer LD** and Hope MJ (1988) "Characterization of Cholesterol Hemisuccinate and Alpha Tocopherol Hemisuccinate Vesicles" Biochim Biophys Acta. 941:165-175.

20. Balazsovits JAE, **Mayer LD**, Bally MB, Cullis PR, Ginsberg RS and Falk RE (1989) "Analysis of the Effect of Liposome Encapsulation on the Vesicant Properties, Acute and Cardiac Toxicities, and Antitumour Efficacy of Doxorubicin" Cancer Chemother. Pharmacol. 23, 81-86.

21. Redelmeier TE, **Mayer LD**, Wong KF, Bally MB and Cullis PR (1989) "Proton Transport in Large Unilamellar Vesicles in Response to Electrical Potentials and Ph Gradients" Biophys. J. 56, 385-393.

22. **Mayer LD**, Tai LCL, Ko DSC, Masin D, Ginsberg RS, Cullis PR and Bally MB (1989) "Influence of Vesicle Size, Lipid Composition and Drug-to-Lipid Ratio on the Biological Activity of Liposomal Doxorubicin" Cancer Research 49, 5922-5930.

23. Cullis PR, **Mayer LD**, Bally MB, Madden TD and Hope MJ (1989) "Generation and Loading of Liposomal Systems for Drug Delivery Applications" Advanced Drug Delivery Reviews 3, 267-282.

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Applications

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7. WO Patent Application # 05102359 (filed 2005) "Combination Formulations of Anthracycline Agents and Cytidine Analogs", Inventors: **Mayer, L.D.**, Johnstone, S., Harasym, T.

8. US Patent Application # 20060165577 (filed 2005) "Compositions for Delivery of Drug Combinations", Inventors: Tardi, P., Harasym, T., Webb, M., Shew, C., **Mayer, L.D.**, Bally, M.B., Janoff, A.S.

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1997- Founder, Director and Chief Scientific Officer, Lipoxen plc

2001- Professor Emeritus, School of Pharmacy, University of London, London

1990-2001: Professor of Experimental Drug Delivery and Head, Centre for Drug Delivery Research, The School of Pharmacy, University of London, London; Medical Research Council Senior Scientist (until Oct. 1993)

1984-1990: Head, Medical Research Council Group and Honorary Senior Lecturer, Academic Medicine, Royal Free Hospital School of Medicine, University of London

1972-1984: Senior Member of Staff; Head, Liposomes Group, Medical Research Council's Clinical Research Centre, Harrow

EXHIBIT A

1970-1972: Research Fellow, Department of Biochemistry, Royal Free Hospital School of Medicine, University of London

1968-1970: Post-Doctoral Research Fellow, Department of Medicine, Albert Einstein College of Medicine, New York

1964-1968: see **Education**

1963-1964: Research Assistant, Allan Memorial Institute of Psychiatry, Montreal

1963: Visiting Scientist, Max Planck Institut fur Kulturpflanzenzuchtung, Hamburg

1960-1963: Research Scientist, Hellenic National Foundation of Research, Athens.

Societies:

- Biochemical Society (since 1971)
- Controlled Release Society (1981-1983); 1994 (life member)
- Harvey Society (1969-1976)
- Hellenic Biochemical and Biophysical Society (since 1975)
- Medical Research Society for Inherited Metabolic Diseases (since 1976)
- U.K. Association of Pharmaceutical Scientists (since 1991)
- American Association of Pharmaceutical Scientists (since 1996; Fellow (since 1998))
- Hellenic Medical Association (since 1997)

Awards/Distinctions:

- Controlled Release Society Founders Award, (1994) "for outstanding contributions to drug targeting and delivery"
- Alec D. Bangham MD, FRS Achievement Award (1995) "for lifelong achievement resulting in a fundamental and sustained impact on the advancement of liposome science and technology"

- Elected to Fellowship status in the American Association of Pharmaceutical Scientists (1998) "for outstanding contributions to pharmaceutical sciences"

- Doctor of Science (DSc) award by the University of London (2001)

- President elect, International Liposome Society (since 2001)
  Acting President, International Liposome Society (1999-2001)

  Journal of Drug Targeting Award (2008) for life-long achievements in liposome research

- Entry in WHO=s WHO (since 1999)

Other awards:

- Gordon Research Conference Grant 1977 (US $5,000) to organize and chair the first Gordon Research Conference on "Drug Carriers in Biology and Medicine" in 1978

- ASI Grant from NATO's Scientific Affairs Division, 1980 (US $40,000) to direct a NATO Advanced Studies Institute in 1981

- ASI Grant from NATO's Scientific Affairs Division, 1982 (US $40,000) to direct a NATO Advanced Studies Institute in 1983

- ASI Grant from NATO's Scientific Affairs Division, 1984 (US $35,000) to direct a NATO Advanced Studies Institute in 1985

- ASI Grant from NATO's Scientific Affairs Division, 1986 (US $35,000) to direct a NATO Advanced Studies Institute in 1987

- ASI Grant from NATO's Scientific Affairs Division, 1987 (US $42,000) to direct a NATO Advanced Studies Institute in 1988

- ASI Grant from NATO's Scientific Affairs Division, 1988 (US $40,000) to direct a NATO Advanced Studies Institute in 1989

- ASI Grant from NATO's Scientific Affairs Division, 1989 (US $55,000) to direct a NATO Advanced Studies Institute in 1990

- ASI Grant from NATO's Scientific Affairs Division, 1990 (US $50,000) to direct a NATO Advanced Studies Institute in 1991

- ASI Grant from NATO's Scientific Affairs Division, 1991 (US $45,000) to direct a NATO Advanced Studies Institute in 1992

- ASI Grant from NATO's Scientific Affairs Division, 1992 (US $45,000) to direct a NATO Advanced Studies Institute in 1993

- ASI Grant from NATO's Scientific Affairs Division,1993 (US $40,000) to direct a NATO Advanced Studies Institute in 1994

- ASI Grant from NATO's Scientific Affairs Division, 1994 (US $45,000) to direct a NATO Advanced Studies Institute in 1995

- ASI Grant from NATO's Scientific Affairs Division, 1995 (US $45,000) to direct a NATO Advanced Studies Institute in 1996

- ASI Grant from NATO's Scientific Affairs Division, 1996 (US $45,000) to direct a NATO Advanced Studies Institute in 1997

- ASI Grant from NATO=s Scientific Affairs Division, 1998 (US $40,000) to direct a NATO Advanced Studies Institute in 1999

- Recipient of exchange visits award ($4,000) by the Anglo-German Foundation of British Council (1990-1992)

- Recipient of exchange visits award ($4,000) by the Anglo-German Foundation of British Council (1993-1994)

Research Support: Research during 1972-1984, (see Research Appointments) was fully supported by the Medical Research Council (MRC)

Subsequent grants include:

- Research Contract (N01-CM-97171) with the National Cancer Institute (USA) 1978 (US $188,000) (1979-1982)

- Medical Research Council project grant (, 95,000; 1984-1987)

- MRC's Clinical Research Centre support (, 100,000, 1984-93)

- Medical Research Council project grant (, 106,000; 1987-1990)

- WHO (, 12,000; 1985)

- Ministry of Defence (, 49,000; 1992-1993)

- Ministry of Defence (, 160,000; 1992-1994)

- Ministry of Defence (, 127,000; 1993-1995)
- Sequus Pharmaceuticals. (, 98,000; 1993-1996)
- Ministry of Defence (, 139,264; 1994-96)
- Ministry of Defence (, 75,000; 1996)
- Ministry of Defence (, 20,000; 1996)
- ATTA, France (, 30,000; 1995-1998)
- European Commission (BRITE/EURAM; 240,000 ECU, 1997-2001)
- European Commission: 176,000 ECU (1997-2000)
- ICI (, 36,000; 1996-1999) to support a Ph.D. studentship
- Glaxo Welcome (, 20,100; 2000-2003) to support a Ph.D. studentship
- Royal Society Joint Project Award (Japan) (, 10,200; 2000-2002)

Other previous support includes grants from The Leverhulme Trust, The World Laboratory, The Royal Society, the Wellcome Trust, Wellcome Biotechnology, WHO, The British Council, The British Technology Group; Speywood Laboratories Ltd; University of London Central Fund

Research and Teaching: Supervision of well over 100 Post-Doctoral workers, Scholars, Visiting Scientists, Post Graduate students, Technicians, Erasmus and Sandwich students working on various aspects of drug targeting with liposomes and other systems. Individuals who worked with Professor Gregoriadis for a minimum of three months are shown below in approximate chronological order (1972-to date):

Rosemary A. Buckland (Technician)
Diane Neerunjun (Technician)
Christopher D.V. Black (Ph.D. student)
Anthony W. Segal (Visiting Scholar)
Gerry Dapergolas (Ph.D. student; Greek Government Scholar)
Isobel Braidman (Post doc)
Pamela J. Davisson (Sandwich student)
Susan Scott (Sandwich student)
George Deliconstantinos (Post doc; Greek Government Scholar)

Peter Bonventre (Visiting scientist)
Daniel Wreschner (Post doc)
Emanuel Manesis (Post doc; NATO Scholar)
Christine Davis (Sandwich student)
Chris Kirby (Research Associate)
Roger Moore (Research Assistant)
Jackie Clarke (Technician)
Judith Senior (Technician, Ph.D. student)
Ann Meehan (Sandwich student)
Mon-Moy Mah (Sandwich student)
Catherine Lemonias (Visiting scientist)
Hishani Weereratne (Ph.D. student)
Pamela Large (Research Assistant)
Jim Mixson (Visiting NIH scientist)
Askin Tümer (Post doc; visiting NATO Scholar)
Barbara Wolff (Post doc)
Natalie Garçon (Post doc)
David Davis (Research Assistant)
Alun Davies (Technician)
Jay R. Behari (Post doc; British Council Scholar)
Steven Seltzer (Visiting Fogarty Scholar)
Y. Pathak (Visiting British Commonwealth Scholar)
Volkmar Weissig (Visiting scientist)
Lloyd Tan (Ph.D. student; Government of Singapore Scholar)
Qifu Xiao (Ph.D. student)
Christine Panagiotidi (Technician)
K.L. Kahl (Visiting scientist)
Christine da Silva (Technician)
Brenda McCormack (Ph.D. student; Research Assistant)
M Yasar Ozden (Visiting NATO Scholar)
Natasa Skalko (Ph.D. student)
Zhen Wang (Ph.D. student)
John Giannios (Ph.D. student)
Dmitry Genkin (Visiting scientist)
Maria Georgiou (Visiting scientist)
Sophia Antimisiaris (Visiting scientist)
Becky J. Ficek (Fulbright Scholar)
Victor Kyrylenko (Visiting scientist; Leverhulme Scholar)
Martin Brandl (Post doc; British Council Scholar)
Dieter Bachmann (Post doc; British Council Scholar)
Mayda Gursel (Ph.D. student)
Sabina Ganter (Erasmus student)
Ishan Gursel (Visiting scientist)
Cecilia D=Antuono (Erasmus student)
Ana Fernandes (Ph.D. student)

Cristina Lopez Pascual (MSc student)
Maria Velinova (Visiting scientist)
Susana Morais (Erasmus student)
Ann Young (Research Assistant)
Yannis Loukas (Research Assistant)
Vassilia Vraka (MSc. student)
Voula Kallinteri (Erasmus student)
Fatima Eraçs (Erasmus student)
Jean Marie Verdier (Erasmus student)
Dimitry Fatouros (Erasmus student)
Veronika Muller (Erasmus student)
Jean-Christophe Olivier (Research Assistant)
Janny Zhang (Ph.D. student)
Roghieh Saffie (Ph.D. student)
Irene Naldoska (Visiting British Council Scholar)
Sudaxina Murdan (Ph.D. student; shared)
Sussi Juul Hansen (Erasmus student)
Anette Hollensen (Erasmus student)
Yvonne Perrie (Ph.D. student; Research Assistant)
Maria Jose Saez Alonso (Erasmus student)
Mercedes Valdes (Erasmus student)
Laura Nasarre (Erasmus student)
Eve Crane (Marshall Scholar)
Brahim Zadi (Ph.D. student; Research Assistant)
Maria E. Lanio (Visiting Cuban Government Scholar)
Gernot Warnke (Visiting scientist)
Elizabetta Casali (Ph.D. student)
Sevtap Velipasaoglu (Visiting Turkish Government Scholar)
Sara Lauria (Erasmus student)
Oulaya Belguenani (Erasmus student)
Isabelle Gyselinck (Erasmus student)
Sigrun Lubke (Erasmus student)
Kent Lau (Ph.D. student; ICI Scholar)
Alejandro Soto (Visiting Cuban Government Scholar)
Yanin Bebelagua (Visiting Cuban Government Scholar)
Steve Yang (Ph.D. student; Taiwan Government Scholar)
Filipe Rocha da Torre Assoreira (Erasmus student)
Paola Genitrini (Erasmus student)
Guoping Sun (Visiting British Council Scholar)
Malini Mital (Ph.D. student)
Michael Schupp (Erasmus student)
Karin Gaimann (Erasmus student)
Mia Obrenovic (Ph.D. student)
Sherry Kittivoravitkul (Ph.D. student; Thailand Government Scholar)
Yoshie Maitani (Visiting Royal Society Scholar)

Irene Papanicolaou (Ph.D. student; Glaxo Scholar) (2000-2001)
Miriam Steur (Erasmus student)
Sanjay Jain(Visiting Scientist; Scholar)
Ioannis Papaioannou (Ph.D. student; Lipoxen Scholar)
Maria Verissimo (Erasmus student)
Bruno da Costa (Erasmus student)
Letizia Flores Prieto (Erasmus student)

Research Interests:

Past and present interests (see list of references) include:

- Metabolism and function of trace metals;
- Recognition groups on glycoproteins, cell receptors and catabolism of proteins;
- Lysosomes and lysomotropic action;
- Fate of liposomes in vivo and control;
- Mechanisms of liposomal drug action;
- Liposome technology;
- Drug delivery with liposomes in enzyme replacement therapy; cancer and antimicrobial therapy; use of liposomes for the immunopotentiation of vaccines (protein and peptide antigens and live microbes); oral administration of vitamins and peptides; drug targeting with liposomes coated with antibodies or glycoproteins to tumours, liver (hepatocytes), lymphocytes and other accessible cells; photoprotective liposomes; DNA vaccination via liposomes
- Polysialic acids as a means to improve the stability, reduce the immunogenicity and antigenicity when relevant, and extend the circulation time of small drugs, peptides, proteins and liposomes
- Polyglycolic/polylactic acid polymers as drug carriers
- Cyclodextrins (as such or entrapped in liposomes) as drug carriers
- Polyhydroxy butyric acid particles as carriers of live microbes

Collaborative Studies:

There have been numerous collaborative studies with both academic and industrial researchers worldwide on a variety of drug delivery and targeting aspects, many of them published (see list of publications, refs:
27, 28, 31, 34, 35, 39, 44, 46, 55, 61, 64, 65, 66, 69, 75, 81, 82, 86, 87, 88, 89, 109, 113, 126, 127, 147, 151, 156, 161, 164, 167, 168, 169, 173, 177, 178, 184, 186, 187, 189, 190, 199, 200, 205, 211, 212, 214, 215, 216, 219, 221, 224, 236, 241, 249, 253, 258, 260, 267, 270, 288, 289, 290, 314)

Patents:

Inventor in granted or pending patents. These include a cluster of patents on **polysialic acids** as a means to prolong the circulation time of drugs, proteins and liposomes; a cluster of patents on **liposome-mediated DNA vaccination**; and patents on **giant liposomes** as carriers of live microbial vaccines; **cyclodextrin-drug complexes** entrapped in photoprotective liposomes; **small liposomes with high drug loading capacity**; liposomes devoid of cationic lipids in **gene therapy** or **DNA vaccination**; a one step method for **high yield-entrapment of taxol** in small liposomes.

Patents on polysialic acids and liposome-mediated DNA vaccines form the basis of the two platform technologies of **Lipoxen plc** (www.lipoxen.com) a drug delivery company founded by Professor Gregoriadis in 1997.

Contribution to the Field of Drug Delivery Systems

The following is a list of key publications in "high-impact" journals that have motivated the application of liposomes in Biochemistry, Immunology, Pharmacology, Therapeutics and Vaccines

- Gregoriadis and Ryman, **Eur.J.Biochem.**, 24: 485-491, 1972 (Fate of protein-containing liposomes injected into rats. An approach to the treatment of storage diseases), and Gregoriadis and Ryman, **Biochem.J.**, 129:123-133, 972 (Lysosomal localization of β-fructofuranosidase-containing liposomes injected into rats. Some implications in the treatment of genetic disorders) (**First papers to study the fate of liposomes in vivo, and demonstrate lysosomal localization. The latter paper also proposed a variety of liposomal uses, including antimicrobial and cancer treatment and gene therapy**).

- Gregoriadis, **FEBS Lett.**, 36: 292-296, 1973 (Drug entrapment in liposomes) (**First paper to study anti-cancer and antimicrobial drug entrapment and liposomal drug fate in vivo**).

- Gregoriadis and Buckland, **Nature**, 244: 170-172, 1973 (Enzyme-containing liposomes alleviate a model for storage disease) (**First paper to demonstrate in a model of lysosomal storage disease that liposomes containing an appropriate enzyme could potentially be used in the treatment of lysosomal storage diseases**).

- Gregoriadis et al, **Lancet**, i: 1313-1316, 1974 (Drug-carrier potential of liposomes in cancer chemotherapy) (**First study of (cancer) patients injected with liposomes**).

- Allison and Gregoriadis, **Nature**, 252: 252, 1974 (Liposomes as immunological adjuvants) **(First paper to demonstrate the immunological adjuvant properties of liposomes for protein antigens. This and subsequent extensive work by the author's group and many others culminated in the production (by Berna) and licencing of liposome-based vaccines against hepatitis A and influenza).**

- Gregoriadis and Neerunjun, **Biochem. Biophys. Res. Commun.**, 65 : 537-544, 1975 (Homing of liposomes to target cells) (**First paper to demonstrate targeting of liposomes to (tumour) cells in vitro (via antibodies against tumours) and in vivo (via a desialylated glycoprotein recognizing the galactose receptor in the liver**).

- Gregoriadis, **New Engl. J. Med.**, 295: 704-710 and 765-770, 1976 (The carrier potential of liposomes in biology and medicine. (Medical Progress article in two parts)) and Gregoriadis, **Nature**, 265: 407-411, 1977 (Targeting of drugs (Review article)) **(First reviews on liposomes and drug targeting respectively which are thought to have helped to attract world-wide attention to the drug delivery potential of liposomes and drug targeting in general by the medical and scientific community. A subsequent Occasional Survey article in the Lancet** (Gregoriadis, **Lancet, ii: 241-247, 1981,** Targeting of Drugs: Implications in Medicine), **also contributed significantly**).

- Belchetz et al, **Lancet**, ii: 116-117, 1977 (Treatment of Gaucher's disease with liposome-entrapped glucocerebroside: β-glucosidase) (**First study of therapeutic use of liposomes in (Gaucher disease) patients**).

- Gregoriadis et al, **Life Sci.**, 21: 357-370, 1977 (Fate of a liposome-associated agent injected into normal and tumour-bearing rodents. Attempts to improve localization in tumour tissues) (**First paper to demonstrate antibody-mediated targeting of liposomes to tumours in vivo**).

- Gregoriadis and Davis, **Biochem. Biophys. Res. Commun.**, 89: 1287-1293, 1979 (Stability of liposomes in vivo and in vitro is promoted by their cholesterol content and the presence of blood cells), and Kirby and Gregoriadis, **Biochem. J.**, 186: 591-598, 1980 (Effect of the cholesterol content of small unilamellar liposomes on their stability in vivo and in

vitro) (**First papers to show the effect of cholesterol on liposomal bilayer stability in plasma or blood and in prolonging vesicle clearance in vivo**).

- Gregoriadis and Senior, **FEBS Lett.**, 119: 43-46,1980 (The phospholipid component of small unilamellar liposomes controls the rate of clearance of entrapped solutes from the circulation) (**First paper to demonstrate that vesicle clearance depends on vesicle phospholipid composition**) (**Together with a paper by Hwang et al published in 1980**). **This and subsequent extensive work by the author's group and others have led to the production (by NeXtar, now Gilead) and marketing of small, long circulating liposomes for the treatment of certain cancers (DaunoXome) and fungal disease (AmBisome).**

- Kirby and Gregoriadis, **Nature Biotechnology**, 2: 979-984, 1984 (Dehydration-rehydration vesicles (DRV): A new method for high yield drug entrapment in liposomes). **(Development of the dehydration/rehydration method. This widely adopted method, ensures high yield entrapment of any water-soluble solute under mild conditions in the absence of sonication or detergents. It has also led to a one step method (J. Drug Targeting,** 3: 469-475, 1996 Gregoriadis et al, High yield incorporation of plasmid DNA within liposomes: Effect on DNA integrity and transfection efficiency) **for the quantitative (>95%) entrapment of DNA within liposomes)**.

- Garçon et al, **Immunology**, 64: 743-745, 1988 (Targeted immunoadjuvant action of tetanus toxoid-containing liposomes coated with mannosylated albumin) (**First paper to demonstrate (mannose-mediated) targeted immunological adjuvant action of liposomes in vivo**).

- Gregoriadis et al, **Immunology**, 80: 535-540, 1993 (Liposome-entrapped T-cell peptide provides help for a co-entrapped B-cell peptide to overcome genetic restriction in mice and induce immunological memory) (**First paper to demonstrate that a T cell epitope can provide help for a B cell epitope to raise an IgG response when the two epitopes are co-entrapped in the same liposome**).

- McCormack and Gregoriadis, **Biochim. Biophys. Acta.**, 1291: 237-244, 1997 (Comparative studies of the fate of free and liposome-entrapped hydroxypropyl-β-cyclodextrin/drug complexes after intravenous injection into rats: Implications in drug delivery) (**First paper to demonstrate that liposomes can control/prolong the pharmacological action of drugs in tissues by entrapping the drug in the form of complexes with cyclodextrins. For instance, liposome-entrapped cyclodextrin-included drugs taken up by the liver remain in the tissue intact and inert until they are dissociated from cyclodextrins. The rate of dissociation depends on the stability constant of the complex, which in turns depends on the types of drug and cyclodextrin used**).

- Gregoriadis et al, **FEBS Lett.**, 402: 107-110, 1997 (Liposome-mediated DNA vaccination) (**First paper to show that liposomes entrapping a plasmid DNA vaccine in the aqueous phase can potentiate immune responses to the encoded antigen to a much greater extent than naked DNA or liposome-DNA complexes**). For more information see: www.lipoxen.com

- Bacon, Caparros-Wanderley, McCormack, Laing and Gregoriadis, CRS Proceedings, 30th Annual Meeting 2003, page 884. (A novel liposomal influenza vaccine) (**First paper to show that co-entrapment in liposomes of a DNA plasmid encoding a vaccine together with the protein vaccine leads to much greater immunity than that seen with either of the antigens entrapped and given separately).**

- **The use of polysialic acids as a means to improve the stability and circulatory life of drugs, peptides, and proteins and also reduce their immunogenicity and antigenicity, was first proposed in 1993** (Gregoriadis et al, **FEBS Lett** 315:271-276, 1993; Polysialic acids: Potential in drug delivery). This publication and subsequent ones (248, 254, 273, 286, 295, 300, 312, 313, 324, 325, 327, 329; see Publications) established polysialic acids as an alternative to PEGylation, especially for peptides and proteins used chronically and in increased amounts. For more information see: www.lipoxen.com

Books:

- Editor, Drug Carriers in Biology and Medicine (Academic Press, 1979)
- Senior Editor (with A.C. Allison), Liposomes in Biological Systems (Wiley, 1980)
- Senior Editor, (with J. Senior and A. Trouet), Targeting of Drugs (Plenum, 1982)
- Editor, Liposome Technology (3 volumes)(CRC Press, Inc., 1984)
- Senior Editor (with G. Poste, J. Senior and A. Trouet), Receptor Mediated Targeting of Drugs (Plenum, 1984)
- Senior Editor (with J. Senior and G. Poste) Targeting of Drugs with Synthetic Systems (Plenum, 1986)
- Editor, Liposomes as Carriers of Drugs: Recent Trends and Progress (Wiley, 1988)
- Senior Editor (with G. Poste) Targeting of Drugs: Anatomical and Physiological Considerations (Plenum, 1988)
- Senior Editor (with A.C. Allison and G. Poste) Immunological Adjuvants and Vaccines (Plenum, 1989)
- Senior Editor (with A.C. Allison) Targeting of Drugs: Optimization Strategies (Plenum, 1990)
- Senior Editor (with A.C. Allison and G. Poste) Vaccines: Recent Trends and Progress (Plenum, 1991)

- Editor, Liposome Technology (3 volumes), 2nd Edition, (CRCPress), 1993

- Senior Editor (with A.T. Florence and H.M. Patel) Liposomes in Drug Delivery, Harwood Academic Publishers, Reading, 1992

- Senior Editor (with A.T. Florence and G. Poste) Targeting of Drugs: The Challenge of Peptides and Proteins (Plenum, 1992)

- Senior Editor (with B. McCormack, A.C. Allison and G. Poste) New Generation Vaccines: The Role of Basic Immunology (Plenum, 1993)

- Senior Editor (with B. McCormack and G. Poste) Targeting of Drugs: Advances in System Constructs, (Plenum, 1994)

- Senior Editor (with B. McCormack and A.C. Allison) Vaccines: New Generation Immunological Adjuvants, (Plenum, 1995)

- Senior Editor (with B. McCormack) Targeting of Drugs: Strategies for Oligonucleotide and Gene Therapy (Plenum, 1996)

- Senior Editor (with B. McCormack and A.C. Allison) Vaccine Design: The Role of Cytokine Networks, (Plenum, 1997)

- Senior Editor (with B. McCormack) Targeting of Drugs: Stealth Therapeutic Systems, (Plenum, 1998)

- Senior Editor (with B. McCormack) Targeting of Drugs: Strategies for Gene Constructs and Delivery, (IOS Press, 2000)

- Guest Editor, J.Drug Targeting (vol 1(1), 1993) Special issue on liposomes as a drug carrier

- Guest Editor, J.Liposome Research (vol 6 (2), 1996) Special issue on liposomal vaccines

- Guest Editor, Int.J.Pharmaceutics (vol 162 (1-2), 1998) Special issue on liposomes as a drug carrier

- Editor, Liposome Technology, 3rd Edition (CRC Press). In press

- Series Co-Editor (with A.T. Florence) of books on Drug Delivery, Harwood Academic Publishers, Reading.

The following books were commissioned and published:

Volume 1	Microencapsulation of Drugs, edited by T.L. Whateley
Volume 2	Liposomes in Drug Delivery, edited by G. Gregoriadis, A.T. Florence and H.M. Patel
Volume 3	Drug Absorption Enhancement: Concepts, Possibilities, Limitations and Trends, edited by A.G. de Boer
Volume 4	Trends and Future Perspectives in Peptide and Protein Drug Delivery, edited by V.H.L. Lee, M. Hashida and Y. Mizushima
Volume 5	Interfacial Phenomena in Drug Delivery and Targeting, edited by G. Buckton
Volume 6	Liposomes in Biomedical Applications, edited by P.N. Shek
Volume 7	Handbook of Biodegradable Polymers, edited by A.J. Domb, J. Kost and D.M. Wiseman
Volume 8	Antigen Delivery Systems: Immunological and Technological Issues, edited by B. Gander, H.P. Merkle and G. Corradin
Volume 9	Submicron Emulsions in Drug Targeting and Delivery, edited by S. Benita
Volume 10	Advanced Gene Delivery, edited by A. Rolland
Volume 11	An Introduction to Niosomes and Other Non-Phospholipid Systems, edited by I. Uchegbu

Journals:

- Editorial Board, Life Sciences (1979-1989)
- Editorial Board, Enzyme and Microbial Technology (1979-1986)
- Editorial Board, CRC Critical Reviews in Therapeutic Drug Carrier Systems (since 1983)
- Advisory Board, Biochemical Journal (1981 and 1982)
- Editor (Regional), Journal of Microencapsulation (1984-1996)
- Advisory Board, Journal of Liposome Research (since 1987)
- Editorial Board, J.Drug Targeting (1992-1999)
- Editorial Board, Artificial Cells, Blood Substitutes and Immobilization Biotechnology (since 1996)
- Editorial Board, J. Tumour Targeting (since 2000)
- Editorial Board, Hellenic Medical Journal (since 2000)
- Editorial Board, Expert Opinion on Therapeutic Patents (since 2000)

- Editorial Board, Current Drug Targeting - Infectious Disorders (since 2000)

Conferences and other activities:

Organizer of 24 international conferences and Summer Schools as shown below:

- Founder, first Chairman and Organizer (1978) of the ongoing Gordon Research Conference Series "Drug Carriers in Biology and Medicine".

- Founder, Director and Organizer of the following NATO Advanced Studies Institute Series on "Drug Targeting":

"Targeting of Drugs" (1981), Cape Sounion Beach, Greece

"Receptor-mediated Targeting of Drugs" (1983), Cape Sounion Beach, Greece

"Targeting of Drugs with Synthetic Systems" (1985), Cape Sounion Beach, Greece

"Targeting of Drugs: Anatomical and Physiological Considerations" (1987), Cape Sounion Beach, Greece

"Targeting of Drugs: Optimization Studies" (1989), Cape Sounion Beach, Greece

ATargeting of Drugs: The Challenge of Peptides and Proteins@ (1991), Cape Sounion Beach, Greece

"Targeting of Drugs: Advances in System Constructs" (1993), Cape Sounion Beach, Greece

"Targeting of Drugs: Strategies for oligonucleotide and gene delivery in therapy@ (1995), Cape Sounion Beach, Greece

"Targeting of Drugs: Stealth Therapeutic Systems" (1997), Cape Sounion Beach, Greece

ATargeting of Drugs: Strategies for Gene Constructs and Delivery@ (1999), Marathon, Greece

- Founder, Director and Organizer of the following NATO Advanced Studies Institute Series "Vaccines":

  "Immunological Adjuvants and Vaccines" (1988), Cape Sounion Beach, Greece

  "Vaccines: Recent Trends and Progress" (1990), Cape Sounion Beach, Greece

  "New Generation Vaccines: The Role of Basic Immunology" (1992), Cape Sounion Beach, Greece

  "Vaccines: New Generation Immunological Adjuvants" (1994), Cape Sounion Beach, Greece

  "Vaccine Design: The Role of Cytokine Networks" (1996), Cape Sounion Beach, Greece

- Symposium Organizer and Chairman (Membranes and Membrane Model Systems). Special FEBS Meeting on "Cell Function and Differentiation" Athens (1982)

- Organizer (with A.T. Florence and H. Patel): "Liposomes in Drug Delivery: 21 Years On", London, 1990

- Organizer (with A.T. Florence): "Liposomes in Drug Delivery: The Nineties and Beyond", London, 1993

- Organizer (with A.T. Florence): "Liposome Advances: Progress in Drug and Vaccine Delivery", London, 1996

- Organizer (with A.T. Florence): ALiposomes Advances: Progress in Drug and Vaccine Delivery@, London, 1999

- Organizer (with A.T. Florence): ALiposomes Advances: Progress in Drug and Vaccine Delivery@, London, 2001

- Organizer: “Liposomes Advances: Progress in Drug and Vaccine Delivery”, London, 2003

- Organizer and Chairman of Controlled Release Society, Symposium on Lipids, Micelles and Liposomes, CRS Conference, San Diego 2001

- Organizer: "Liposome Advances: Progress in Drug and Vaccine Delivery", London, 2005

  Organizer: "Liposome Advances: Recent Trends and Progress in Drug and Vaccine Delivery", London, 2006

  Chairman, Organizing Committee: "Liposome Advances: Recent Progress in Drug and Vaccine Delivery", London, 2007

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Member, Institute for International Advancement of Sciences: 1975-1977

- Member of Advisory Committee, NATO Scientific Affairs Division (from 1982)
- NATO Consultant, P.O. Infections Project, Portugal (1993-1998)
- Lecturer in numerous teaching courses nationally and internationally
- Faculty member (lecturer) of numerous seminars for Industrial staff
- Scientific Consultant (freelance) to numerous pharmaceutical industries
- Invited contributions to **Nature** (one major review and five "News and Views" articles or Reports); **Lancet** (Occasional Survey); **New Engl.J.Med**. (Medical Progress article in two successive issues); **Science** (invited letter); **Pharmacology and Therapeutics** (Review); **Clinical Immunology Newsletter** (Review); **New Scientist** (Review Article); **Drugs** (one Leading Article and one Review); **Trends in Pharmacological Sciences** (Review); **Pharmacy International_** (Review); **Trends in Biotechnology** (three Reviews); **News in Physiological Sciences** (Review); **Immunology Today** (one review; two meeting reports); **Pharmaceutical Research** (Review), and many others (see attached bibliography)

Publications:

- Over 330 publications (see attached list)
- Sole Editor or Senior Editor of 28 volumes (see attached list)
- Numerous conference abstracts

Invited Lectures:

- Over 250 invited lectures (see attached list)

Other activities:

- British Council Lecturer (1983, 1984, 1985, 1987, 1989)
- Numerous book reviews.
- Reviewer for numerous Journals

Lipoxen Technologies Ltd Founder (1997) and Scientific Director of Lipoxen Ltd (now Lipoxen Technologies Ltd), a drug delivery company with laboratories at the School of Pharmacy, University of London, and offices at Suite 303, Hamilton House, Mabledon Place, London WC1H 9BB (www.lipoxen.com)

BOOKS

1. Drug Carriers in Biology and Medicine (G. Gregoriadis, ed.), Academic Press, 1979

2. Liposomes in Biological Systems (G. Gregoriadis and A.C. Allison, eds.), Wiley, 1980

3. Targeting of Drugs (G. Gregoriadis, J. Senior and A. Trouet, eds.), Plenum,1982

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Drug Delivery Systems and Sciences, Vol 2, No 4, pp. 91-97, 2003

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Polysialylated insulin: synthesis, characterization and biological activity in vivo
Biochim.Biophys.Acta, 1622:42-49, 2003

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The effect of polysialylation on the immunogenicity and antigenicity of insulin: Implications for its *in vitro* and *in vivo* activity
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A novel liposomal influenza vaccine
In: Proceedings of 30th Annual Meeting of the Controlled Release Society, p 884

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The Drug Delivery Companies Report, 44-47, 2003

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Polysialylation: the natural way to improve the stability and pharmacokinetics of protein and peptide drugs
Drug Delivery Systems and Sciences, vol 4, No 1, 3-9, 2004

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Introduction: The Origins of Liposomes: Alec Bangham at Babraham
Methods in Enzymology, vol 391, In Press, 2005

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Improving the therapeutic efficacy of peptides and proteins: A role for polysialic acids.
International J.of Pharmaceutics 300, 125-130, 2005

332. G. Gregoriadis, A. Bacon, B. McCormack, P.Laing, B.Frisch and F.Schuber
Liposomebased DNA/protein vaccines: Procedures for entrapment and immunization studies
In: "Liposome Technology" 3rd Edition, Ed. G. Gregoriadis, Informa Healthcare, New York , London, Vol. 3, pp. 233-244, 2007

INVITED LECTURES (Gregory Gregoriadis)

1972

Possible use of Liposomes in Enzyme Replacement Therapy.
Tenth Symposium of the Society for the Study of Inbon Errors of Metabolism, Cardiff

The lysosomotropic action of liposomes
Lysosome Club Meeting, Clinical Research Centre, Harrow, UK

1973

Enzyme or Drug Entrapment in Liposomes: Possible Biomedical Applications
Symposium of Insolubilised Enzymes, Mario Negri Institute for Pharmacological Research, Milan

1974

Liposomes as carriers of drugs in cancer therapy
M.R.C. Toxicology Unit, Carshalton, UK

Structural requirements for the specific uptake of macromolecules and liposomes by target cells. Workshop on cell biological and enzymological aspects of the therapy of lysosomal storage diseases. Leiden, Netherlands

Liposomes as drug vectors
Gordon Conference on Lysosomes, N.H., USA

The delivery of drugs in liposomes
Medical Research Club Meeting, Clinical Research Centre, Harrow, UK

Micro-encapsulated drug therapy
Department of Nephrology, St Peter's Hospital, London

The drug-carrier potential of liposomes. MRC Cyclotron Unit, Hammersmith Hospital, Hammersmith, London

Liposomes as carriers of drugs and enzymes
Novo Pharmaceutical Industry, Copenhagen, Denmark

1975

The carrier potential of liposomes in cancer chemotherapy
9th International Congress of Chemotherapy, London

Homing of liposomes to target cells
Joint Colloquium of the Lipid Group and Dutch Lipid Group on Interaction between Cells, Liposomes and Micelles. University of Liverpool, UK

Drug therapy with liposomes
Pfizer Pharmaceutical Industry, Kent, UK

Role of liposomes in disease and preventive medicine
Lysosome Club Meeting, Medical College of St Bartholomew's Hospital, London

The use of modified liposomes for the development and therapy of model diseases of glycoconjugate catabolism in specific cells. 3rd International Symposium of Glycoconjugates: Functions in Animals, Brighton

Liposomes as carriers of enzymes
Workshop on Enzyme Engineering, Poona, India

1976

Liposomes as carriers of asparaginase in the treatment of leukemia
Royal Postgraduate Medical School, M.R.C. Leukemia Unit, Hammersmith, London

Liposomes as carriers of drugs
Merrell Pharmaceutical Industry, Strasbourg

Liposomes as carriers of drugs and enzymes
Department of Biochemistry, University of Manitoba, Manitoba

Liposomes as carriers of therapeutic agents
Research Institute, The Hospital for Sick Children, Montreal

Enzyme therapy with liposomes
Montreal Children's Hospital, Research Institute, Montreal

Direction of liposome-associated therapeutic agents to target areas in the body
Symposium on Bioactivation and Controlled Drug Release, Stockholm

Liposomes as enzyme carriers in enzyme replacement therapy
Development and Metabolic Neurology Branch, N.I.H. Bethesda

The enzyme- or drug-carrier potential of liposomes in medicine
Department of Biochemistry, University of Ottawa, Ottawa

The carrier potential of liposomes in medicine
The Wellcome Research Laboratories, Burroughs Wellcome Co., North Carolina

Organ targeting by surface modification of drug-containing liposomes
Department of Pharmacy, The University of Aston in Birmingham, Birmingham

Liposomes as carriers for pharmacologically active agents
School of Engineering and Science, The Polytechnic of Central London, London

Liposomes as immunological adjuvants
The National Bacteriological Laboratory, Stockholm

Enzyme-carrier potential of liposomes. Possible Therapeutic Applications
Department of Biochemistry, McGill University, Montreal

Liposomes as carriers of therapeutic agents
Boehringer, Mannheim

Liposomes as carriers of enzymes in therapy
Meeting of the Association of Clinical Biochemistry, The Postgraduate Medical Centre, Guildford

Liposomes as immunological adjuvants
Department of Immunology, The Middlesex Hospital Medical School, London

1977

Liposome-encapsulation of enzymes for therapy
Symposium on Pharmaceutical and Medical Aspects of enzyme Biotechnology, London

Liposomes as drug delivery systems
Department of Pharmacy, University of Nottingham, Nottingham

Liposomes
School of Natural Sciences, The Hatfield Polytechnic, Hatfield

Liposomes as carriers of pharmacologically active agents
Pharmaceutical Division, Imperial Chemical Industries Ltd., Cheshire

The carrier potential of liposomes in biology and medicine
Roche, N.J.

Liposomes in Medicine
Alza Corporation, Palo Alto

Liposomes as carriers of drugs and enzymes
A and M University, Department of Chemistry, College Station, Texas

Liposomes in biology and medicine
Merck, N.J.

Liposomes as a carrier for the oral administration of insulin
Searle, Chicago

The drug carrier potential of liposomes in cancer chemotherapy
Macromolecular Institute, Midland, USA
Liposomes as carrier of enzymes
Annual meeting of the International Federation of Institutes for the Advancement of Science, University of Pennsylvania, Philadelphia

Liposomes in therapeutic and preventive medicine
Conference on Liposomes, N.Y. Academy of Sciences, New York

The drug carrier potential of liposomes in medicine
Albert Einstein College of Medicine, Bronx, N.Y.

Liposomes in medicine
Smith Kline French, Philadelphia

Liposomes as carriers of proteins: Possible medical applications
Enzyme Engineering Meeting, Bad Neuenahr

1978

Liposomes as carriers of drugs in medicine: Current realities
Yale University, Department of Nuclear Medicine, New Haven

Liposomes: Properties and Applications, Exxon, N.J.

Drug Targeting: Molecular, cellular and synthetic systems and possible co-operation

1st Gordon Conference on Drug Cancer in Biology and Medicine
N.H. USA

A possibility for oral insulin?
17th Congress of European Paediatric Endocrinology, Athens

Interaction of liposomes with the liver
Autumn meeting of the British Society of Gastroenterology, Edinburgh

Liposomes in medicine: the development of the drug-carrier potential
Symposium on "Liposomes" Battelle, Geneva

A role for liposomes in the treatment of malaria?
WHO Workshop on the chemotherapy of malaria, Geneva

Liposomes and their uses in biology and medicine
Department of Surgery, St. George's Hospital Medical School

1979

Insulin and liposomes
Laboratoire Inserm Hopital Herold, Paris

Liposomes in biology and medicine
Société de Belgique, Brussels

Long term experiences in treatment of type I Gaucher's disease with liposome -entrapped glubocerebroside:β-glucosidase
2nd International Symposium in Enzyme Therapy of Genetic Diseases, National Foundation - March of Dimes, South Carolina

Possible clinical application of liposomes
Department of Chemistry and Molecular Sciences, University of Warwick

Liposomes as drug carriers: recent developments
Department of Biochemistry, University College, London

Perspectives in the use of liposomes in therapy
Boerhaave Course: Cell Biological Aspects of Disease. The plasma-membrane and liposomes. University of Leiden, Leiden

Liposomes as a transport mechanism at the cellular and subcellular level
Plenary lecture at the XIth International Congress of Biochemistry, Toronto

1980

Stability of liposomes in vivo and in vitro: Implications for therapeutic use
2nd Gordon Conference on Drug Carriers in Biology and Medicine. N.H. USA

Liposomes and hepatitis B vaccine
2nd Gordon Conference on Drug Carriers in Biology and Medicine. N.H. USA

Liposomes as immunological adjuvants for hepatitis B surface antigens
Symposium on "Liposomes and Immunobiology", University of Texas at Houston, Houston

Targeting of liposomes in vitro and in vivo.
Plenary lecture at the 13th FEBS meeting, Jerusalem

Experience and perspectives of liposomes in medicine
7th International Forum on Subcellular Methodology, Guildford

Liposomes as a drug delivery system.
Symposium on "Liposomes in the study of drug activity and immunocompetent cell functions" Station de Recherches de Virologie and d'Immunologie, Thiverval-Grignon, France

Control of liposomal stability in vivo
Department of Medical Biochemistry, A and M University, Texas

Long-term treatment of Gaucher's disease Type I with liposomal glycocerebrosidase
Department of Clinical Research, Scripps Clinic and Research Foundation, San Diego

Cancer chemotherapy with liposomes. Control of drug release from liposomes in vivo.
National Cancer Institute, Bethesda

1981

Drug Targeting
National Institute for Biological Standards and Control, London

Control of liposomal drug release and clearance after parenteral administration
Centre de Biophysique Moleculaire, Orléans

Liposomes as drug delivery systems
International Conference on Drug Delivery System Development, London

Targeting of drugs with liposomes: Studies on optimization
17th Symposium on "Optimization of drug delivery". Alfred Benson Fond, Copenhagen

Targeting of drugs. A review of potential protein drug carriers
NATO Advanced Study Institute on "Targeting of Drugs", Cape Sounion Beach, Greece

Targeting of drugs with liposomes. Factors influencing effective action
NATO Advanced Study Institute on "Targeting of Drugs", Cape Sounion Beach, Greece

Controlled release of drugs from liposomes in vivo
8th International Controlled Radioactive Materials Symposium. Ft Lauderdale, Florida

Liposomes in enzyme therapy of Gaucher's disease
2nd Clinical Research Centre Symposium on "Advances in the Treatment of Inborn Errors of Metabilism", Harrow, UK

Liposomes in enzyme therapy of Gaucher's disease. A possible experimental model.
1st International Symposium "Gaucher's Disease: The most prevalent Jewish Genetic Disease", Mount Sinai School of Medicine, New York

Liposomes as drug carriers
41st International Congress of Pharmaceutical Sciences, Vienna

Targeting of Drugs
"Teach in" for medical journalists. Royal Society of Medicine. London

Control of fate and behaviour of liposomes in vivo
European Science Foundation workshop on synthesis, properties and application of ordered systems in polymer chemistry and life science. Mainz, Germany

Liposomes as adjuvants
MRC Committee on Development of Vaccines and Immunization procedures.

Recent progress in liposomes research
Academisch Ziekenhuis, University of Amsterdam

Targeting of liposomal drugs: Important prerequisites for success
Brunel University, School of Biological Sciences

1982

Liposomes as adjuvants in hepatitis B vaccine
MRC CDVIP working party group on hepatitis vaccine

Liposomes as a drug delivery system
World Symposium: Better protection of pharmaceutical innovation, Paris

Drug Targeting with liposomes
University of Bologna, Istituto di Patologia, Bologna

Control of fate and behaviour of liposomes in vivo
Plenary lecture, Special FEBS Meeting, Athens

Drug Targeting
2nd Symposium on Antimicrobial Chemotherapy, London

Liposomes in vivo
3rd Gordon Conference on Drug Carriers in Biology and Medicine. N.H., USA

"Liposomes in haematology: Tools for research and clinical application"
19th Congress of the International Society of Haematology, Budapest

Optimization of liposome behaviour in vivo
International Conference on Liposomes in vivo, Grignon, France

1983

Control of liposomes in vivo leading to targeting
NATO ASI "Receptor-Mediated Targeting of Drugs", Cape Sounion Beach, Greece

The galactose receptor
NATO ASI "Receptor-Mediated Targeting of Drugs", Cape Sounion Beach, Greece
Liposomes for drug delivery
Hellenic Pharmacological Society, Athens, Greece
Liposomes as drug carriers: Conditions for in-vivo targeting
186th American Chemical Society Meeting (Plenary lecture), Washington, D.C.

Targeting of liposomes in vivo
Merck, Sharp Dohme, Inc. Rahway, N.J.

Control of liposome fate in vivo: Prerequisites for targeting
Biochemical Society Symposium "Medical Application of Liposomes" (Plenary lecture), Charing Cross Hospital Medical School, London

Use of liposomes as enzyme carrier for the treatment of enzyme deficiencies
Centre de Biophysique Moleculaire, Orléans, France

Behaviour of liposomes in vivo: Control leading to targeting
Max Planck Institut für Biochemie, Munich, Germany

1984

Targeting of liposomes in vivo. Prerequisites for success
The University of Aston in Birmingham, Birmingham, UK

Liposomes as a drug delivery system
Boehringer Mannhein GmbH, Tutzing, Germany

Targeting of liposomes in vivo
New York Academy of Sciences. Conference on macromolecules as drugs and as carriers for biologically active materials

A simple method for high yield entrapment of drugs into liposomes in the absence of organic solvents, sonication and detergents
4th Gordon Conference "Drug Carriers in Biology and Medicine", USA

Liposomes as drug carriers
Parenteral Society, Autumn meeting Slough/Windsor, UK

Liposomes as carriers of drugs and vaccines
Bioscience Futures Conference (Online), London, UK

Dehydration/rehydration vesicles: A simple method for high-yield entrapment in liposomes
International Symposium "Medical Application of Liposomes", Nagoya, Japan

Liposomal stability in and clearance from the blood: Optimization leading to targeting in vivo
International Symposium "Medical Application of Liposomes", Nagoya, Japan

Possibilities for liposomes as a drug carrier
Daiichi Seiyaku Co. Ltd., Tokyo, Japan

Recent developments in liposome research.
Organising Scientific Development Group. Oss, Holland

1985

Liposomes: The myth and the reality
Pfizer Central Research, Sandwich, UK

Recent progress in liposome research: Implications in medicine
Université Libre de Bruxelles Institut Jules Bordet, Brussels, Belgium

Control of liposome fate in vivo
International Institut of Cellular and Molecular Biology (ICP) Brussels, Belgium

Liposomes in vivo: Recent progress in controlling their fate
Inserm, Leon Berard, Lyon, France

Fate of liposomes in vivo

NATO ASI "Targeting of Drugs with Synthetic Systems", Cape Sounion Beach, Greece

Dehydration-rehydration vesicles (DRV): High yield entrapment under mild conditions
NATO ASI "Targeting of Drugs with Synthetic Systems", Cape Sounion Beach, Greece

Control of liposomes in vivo
Kali-Chemie Pharma, Hannover

Targeting of drugs: The future
Universidad del Pais Vasco, Bilbao

Liposomes in biological systems
University of Murcia, Spain

1986

Liposomes as a drug delivery system: Optimization of behaviour in vivo
Symposium "New Technological Applications of Lipid Bilayers", Tenerife

Encapsulation of enzymes and other proteins in liposomes
Royal Society of Chemistry, Symposium "Chemical Aspects of Food Enzymes", University of Reading, Reading

Liposome technology and applications
School of Pharmacy, London University, Brunswick Square, London
Liposomes in cancer treatment and prevention
Symposium "Lipids and Cancer", Royal Society of Medicine, London

Possibilities for liposomes in cancer therapy
International Symposium "Ether Lipids in Oncology", Deutsche Krebsgesellschaft, Gottingen

Liposomes as immunological adjuvants for vaccines
Wellcome Biotechnology, Beckenham, Kent

1987

Fate of liposomes after intravenous injection: Control of stability and clearance
CIBA Geigy, Horsham, UK

Liposomes as Immunological Adjuvants: Possibilities for control
NATO ASI "Targeting of Drugs: Anatomical and Physiological Considerations", Cape Sounion Beach, Greece

Technology of liposomes as immunological adjuvants

NATO ASI "Targeting of Drugs: Anatomical and Physiological Considerations", Cape Sounion Beach, Greece

Liposomes as immunological adjuvants: The nature of immune response to entrapped antigens and the role of liposomal characteristics
Halle Liposome Symposium, Halle, Germany

Immunoadjuvant action of liposomes: Optimization studies
Gordon Research Conference "Liposomes and other Organized Lipid Assemblies" New London, USA

Liposomes as Drug Carriers
International Congress of Microencapsulation, Dubrovnic, Yugoslavia

Drug Delivery Systems
IV Mediterranean Congress on Chemical Engineering, Barcelona, Spain

1988

Liposomes for targeted drug delivery
Symposium "Le Medicaments de l'an 2000", Foundation Universitaire des Sciences et Techniques du Vivant, Annecy, France

Liposomes as carriers of drugs and vaccines
Ninth European Immunology Meeting, Satellite Symposium on Immunology and Biotechnology, Rome

Liposomes and the brain
11th Annual Scientific Meeting of the Canadian College of Neuropsychopharmacology, Montreal

Liposomes as immunoadjuvants
UCLA Symposium "Liposomes in the Therapy of Infectious Diseases and Cancer", Lake Tahoe, USA

Liposomes as immunological adjuvants: Optimization Studies
NATO ASI "Immunological Adjuvants and Vaccines", Cape Sounion Beach, Greece

Liposome Technology
NATO ASI "Immunological Adjuvants and Vaccines", Cape Sounion Beach, Greece

1989

Liposomes as drug carriers: Recent trends and perspectives
Department of Biological Sciences, Keele University, Keele

Targeting of drugs: A role for liposomes

Hellenic Medical Society, Royal Postgraduate Medical School, Hammersmith, London

Biological behaviour of liposomes
5th International Colloquium on Lecithin, Cannes

Liposomes as immunological adjuvants: Role of structural characteristics and entrapped mediators
NATO ASI "Targeting of Drugs: Optimization Strategies", Cape Sounion Beach, Greece

Liposomal surface charge and clearance from the circulation: Reevaluation of the status quo
NATO ASI "Targeting of Drugs: Optimization Strategies", Cape Sounion Beach, Greece

Liposomes as drug carriers
Plenary lecture, 19th FEBS Meeting, Rome

Liposomes as immunological adjuvants in vaccines
Third Meeting on Membrane Biotechnology, A and M University, Station College,Texas

Liposomes in immunopotentiation
33rd Harden Conference: "Cellular Barriers and Drug Targeting", Wye College, Ashford, Kent

Liposomes: Problems and prospects
33rd Harden Conference: "Cellular Barriers and Drug Targeting", Wye College, Ashford, Kent

1990

Liposomes in the treatment of cancer: Past, present and future
1st International Conference on "Platinum Complexes and Liposomes in the treatment of Cancer", Barcelona

Liposomes: 21 years of progress
7th International Symposium on Microencapsulation, Glasgow

Liposomes as carriers of drugs and vaccines
First World Medical Conference of Greek Diaspora, Athens

Introduction on Drug Targeting: APhysiologic Mechanisms and Pathologies Involved in Drug Targeting and Imaging" (Conference Co-organiser), Compiegne, June

2nd NATO Advanced Studies Institute on "Vaccines: Recent Trends and Progress" (ASI Director and Lecturer), Cape Sounion Beach, Greece

Liposomes: Structure and Properties
X Corso Avanzato di Chimica Pharmaceutica, Bressanose

Liposomes: Recent Progress
1st International Conference on Drug Delivery, Barcelona

Liposomes as immunological adjuvants in vaccines
International Symposium on "Liposomes in Biology and Medicine", Tashkent, Uzbekistan

Drug Delivery: Liposomes and Immunoliposomes
MRC AIDS Directed Programme: "New approaches towards the use of oligonucleotides as anti-HIV agents", The Royal Marsden Hospital, London

Liposomes as immunological adjuvants for peptide and protein antigens
Dept of Immunology, University College and Middlesex School of Medicine, London

Preparation of small liposomes with improved drug entrapment yield
International Conference, Liposomes in Drug Delivery: 21 Years On, London

Liposomes as immunoadjuvants for protein and peptide antigens
International Conference, Liposomes in Drug Delivery: 21 Years On, London

1991

Overview of Liposomes
Symposium on Liposomal Amphotericin B (AmBisome) in the Treatment of Systemic Fungal Infection; Stratford-on-Avon, UK

Liposomes: A new-generation of immunological adjuvant
Texas A and M University, Dept of Molecular Biology, College Station, Texas

Liposomes as immunological adjuvants for protein and peptide antigens
NATO ASI "Targeting of Drugs: The Challenge of Peptides and Proteins", Cape Sounion Beach, Greece

Drug incorporation into liposomes: Recent progress
NATO ASI "Targeting of Drugs: The Challenge of Peptides and Proteins", Cape Sounion Beach, Greece

Liposomes
European Continuing Education College "Microencapsulation of Drugs", Liverpool

Liposome research: Vaccine and drug delivery
ULLA Staff meeting, Noordwijkerhout

The future of liposomes - Liposomes of the future
International Conference on Liposome Dermatics; Bad Griesbach, Germany

Use of liposomes in drug delivery
Roussel Uclaf, Romainville, France

Liposomes as topical carriers: Work in Progress
IV International Meeting on Cosmetic Dermatology, Progress in Cosmetic Dermatology: Science and Safety, Rome

Liposomes: Structure, behaviour in vivo and applications
Third International Conference on Drug Delivery and Targeting Systems: Prospects for the 90's, London

Liposomes as carriers of drugs and vaccines
Pharmaceutical Institute, University of Freiburg, Freiburg

The use of liposomes as immunological adjuvants in vaccines
Second Anglo-Egyptian Conference of Pharmaceutical Sciences, Alexandria

1992

New generation of immunological adjuvants: A role for liposomes
UMDS Guy's and St Thomas's Medical and Dental School, Division of Immunology, London

"Liposome Research Days": Chairman= Introduction, Leiden

Liposomes as immunological adjuvants
NATO ASI "New Generation Vaccines: The role of Basic Immunology", Cape Sounion Beach, Greece

Liposome technology as applied to vaccines
NATO ASI "New Generation Vaccines: The role of Basic Immunology", Cape Sounion Beach, Greece

Use of liposomes in biotechnology
"Mediterranean Universities School for Biotechnology", Ankara

Liposomes and polysaccharides as drug delivery systems
Royal Society of Chemistry Symposium "Encapsulation and Controlled Release", 14-
Manchester

1993

Targeting of drugs
Institute of Pharmaceutical Research and Technology, Athens

Targeting of drugs: Liposomes and other Systems (Two lectures)
National Centre for Scientific Research "Demokritos", Athens

Liposomes as drug carriers: A historical perspective
NATO ASI "Targeting of Drugs: Advances in System Constructs", Cape Sounion Beach, Greece

Liposome Technology
NATO ASI "Targeting of Drugs: Advances in System Constructs", Cape Sounion Beach, Greece

Phospholipids vesicles (liposomes) in drug targeting
European Research Conference on Interfaces and Colloidal Systems, York, UK

Liposomes as immunological adjuvants for proteins and peptide antigens
Defence Research Liposome Workshop, Toronto

Liposomes as immunological adjuvants
Chemical and Biological Defence Establishment, Porton Down, UK

Use of liposomes as immunological adjuvants for proteins and peptide vaccines
Liposomes in Drug Delivery: The Nineties and Beyond, School of Pharmacy, London

1994

Drugs-in cyclodextrins-in liposomes: A novel concept in drug delivery
Université Paris-Sud, Centre d'Etudes Pharmaceutiques, Paris

Overview of liposomes
Symposium: Clinical Impact of Liposomal Amphotericin (AmBisome), Royal Society of Medicine, London

Drug targeting and delivery: Recent trends and progress
7th Panhellenic Pharmaceutical Congress, Athens

Liposomes as immunological adjuvants and carriers for peptides, proteins and particulate antigens: An overview
NATO ASI "Vaccines: New-Generation Immunological Adjuvants", Cape Sounion Beach, Greece

Liposome Technology
NATO ASI "Vaccines: New-Generation Immunological Adjuvants", Cape Sounion Beach, Greece

Targeting of drugs (Six lectures)
University of Coimbra, Department of Pharmacy, Coimbra

Liposomes and the development of vaccines for the 21st century
3rd World Bio-Medical Conference of the Hellenic Diaspora, Athens

1995

Liposomes as carriers of peptide, proteins and microbial vaccines
5th International Symposium on Delivery and Targeting of Peptides, Proteins and Genes, Leiden, 17-20 May

Novel liposome-DNA Constructs: In vitro and in vivo studies
NATO ASI Targeting of Drugs: Strategies for Oligonucleotides and Gene Delivery in Therapy, Cape Sounion Beach, Greece, 24 June - 5 July

Effective nucleic acid incorporation within submicron and micron size liposomes under wild conditions, NATO ASI Targeting of Drugs: Strategies for Oligonucleotides and Gene Delivery in Therapy, Cape Sounion Beach, Greece, 24 June - 5 July

A.D. Bangham MD, FRS Achievement Award Lecture
Fouth Liposome Research Days Conference, 30 August-2 September

Liposomes as immunoadjuvants and vaccines
Commet course: Liposome Technology-Clinical and Industrial Applications, Lisbon, 14-19 September

High yield incorporation of plasmid DNA within liposomes: effect on DNA integrity and transfection efficiency
School of Pharmacy, Research Day, London, 22 November

Liposomes as carriers of vaccines
6th Princeton Liposome Conference: Cellular Signals, Clinical Trials and Gene Therapy, Princeton, 28-29 November

Liposomes in drug and vaccine delivery
Symposium on Recent Advances in Drug Delivery Techniques and Testing, Bombay, 11-12 December

1996

Liposomes in immunopotentiation: The co-adjuvant action of interleukins
NATO ASI Vaccine Design: The Role of Cytokine Networks, Cape Sounion Beach, Greece, 24 June - 5 July

Incorporation of antigens and cytokines into liposomes

NATO ASI Vaccine Design: The Role of Cytokine Networks, Cape Sounion Beach, Greece, 24 June - 5 July

Liposome-entrapped vs liposome-complexed and naked plasmid DNA: Comparative studies on DNA vaccination
Liposome Advances: Progress in Drug and Vaccine Delivery, London, 16-20 December

1997

Liposome-mediated DNA vaccination
Dept of Immunology, The Medical College of St Bartholomew=s Hospital, London, 11 February

A potential role for liposomes in DNA vaccination
16th Pharmaceutical Technology Conference, Athens, Greece, 15-17 April

Liposome-DNA formulations
International Working Group on the Standardization and Control of Nucleic Acid Vaccines, National Institute of Biological Standards and Control, South Mimms, Herts, 9 May

Polysialylated enzymes: Increased stability and half-lives in the circulation
NATO ASI Targeting of Drugs: Strategies for Stealth Therapeutic Systems, Cape Sounion Beach, Greece, 24 June - 5 July
Polysialylation of enzymes and liposomes
NATO ASI Targeting of Drugs: Strategies for Stealth Therapeutic Systems, Cape Sounion Beach, Greece, 24 June - 5 July

Applications of liposomes and related technolgoy
Galenos III Intensive Course, Patras, Greece, 6-13 July

Liposomes and immunopotentiation of vaccines
Galenos III Intensive Course, Patras, Greece, 6-13 July

DNA vaccination: A role for liposomes
Galenos III Intensive Course, Patras, Greece, 6-13 July

Polysialic acids: A novel approach to drug delivery
Polysaccharide Biotechnology, National Centre for Macromolecular Hydrodynamics, Sutton Bonington, UK, 3-5 September

Liposome-Technology: Dehydration-rehydration vesicles and their application in drug and vaccine delivery
2nd Central European Symposium on Pharmaceutical Technology, Portoroz, Slovenia, 25-26 September

Liposome-entrapped DNA: A role in DNA vaccination
Third Annual Genetic Vaccines IBC Conference: Genetic Vaccines and Immunotherapeutic Strategies, Orlando, Florida, 16-18 September

Liposome-mediated DNA vaccination
Medical Applications of Biotechnology, Havana, Cuba, 1-6 December

1998

Drug Carriers in the next millenium (Keynote Lecture)
Gordon Research Conference, Drug Carriers in Biology and Medicine, Ventura, CA, 22-27 February

Liposome as carriers of drugs and vaccines
The Hatter Institute and Centre for Cardiology, University College Medical School, London, 19 March

Genetic vaccines: Liposome-based strategies for optimization
Research Day, School of Pharmacy, London, 2 April

Genetic vaccines: Strategies for optimization
2nd International Meeting on Pharmacy and Pharmaceutical Sciences, 6-9 September,
Istanbul

Liposomes supremolecular structures as drug delivery vectors
Conference on New Advances in Drug Delivery Systems, 13-14 October, London

Liposome-mediated DNA vaccination
European Commission meeting on Gene Therapy, 18-20 October, Coimbra

Liposomes in gene delivery
ADemocritos@ Nuclear Research Centre, 26 October, Athens

Optimissing cationic liposome-mediated DNA immunization
IIR Conference on Novel Viral and Non-Viral Gene Delivery Systems, 16-17 November, London

1999

Genetic vaccines: Optimization with DNA delivery systems
Advances in Technology & Business Potential of New Drug Delivery Systems
CRS, Goa, India, 19-20 February

Strategies for the optimization of DNA vaccine delivery
Drug Delivery Systems, IIR Ltd, London, 30 March

International Working Group on the Standardization and Control of Nucleic Acid Vaccines - NAVSaC V, South Mimms, 29 March

Liposome-based vaccines
European Perspectives in the Control of Infectious Diseases; organized by Erasmus Universiteit Rotterdam, Malta, 16 April

Genetic Vaccines: Strategies for optimization
NATO Advanced Studies Institute, Targeting of Drugs: Strategies for Gene Constructs and Delivery
24 June - 5 July 1999, Marathon, Greece, 24 June - 5 July

Liposomes as carriers of DNA vaccines
NATO Advanced Studies Institute, Targeting of Drugs: Strategies for Gene Constructs and Delivery
24 June - 5 July 1999, Marathon, Greece, 24 June - 5 July

Liposome-based DNA vaccines
Protein and Gene-Based Drugs: Product Development and Delivery Challenges (workshop)
American Association of Pharmaceutical Scientists, Annual Meeting
New Orleans, 14-18 November

Liposome-mediated DNA immunization improves humoural and cell-mediated responses
4th International Conference ALiposome Advances: Progress in Drug and Vaccine Delivery@
School of Pharmacy, London, 13-19 December

2000

Liposome-mediated DNA Vaccination: Recent Progress
WHO, Geneva 23-24 May

Liposome-based DNA Vaccines
Symposium on DNA vaccines, gene therapy, and antisense oligonucleotides
Brno, Czech Republic, 18-19 May

DNA vaccines: A role for liposomes
IIR Conference on Proteins, Peptides and Drug Delivery
London, 19-20 July

Liposomes: Structural characteristics and behaviour in vivo
ECIS, University of Patras, Greece, 17-22 September

Liposome-based DNA vaccines
ECIS 2000; University of Patras, Greece, 17-22 September

DNA vaccines: A role for liposomes
Dept of Pharmaceutics, Johan Wolfang Goethe University, Frankfurt, October

2001

DNA vaccines
IIR, 9th Annual Drug Delivery Conference, Brighton, 10-11 May

Liposome-based DNA vaccines
CRS Symposium on Lipids, Liposomes and Micelles, San Diego, 23-27 June

Improving the stability and pharmacokinetics of drugs
Drug Delivery 2001: Next Generation Technology, The Hatton, London, 1-2 October

Liposomes: Structural characteristics and optimization of behaviour in vivo
Aston School of Pharmacy, Aston University, 24 October

DNA vaccines: A role for liposomes
5th International Conference ALiposome Advances: Progress in Drug and Vaccine Delivery
School of Pharmacy, London, 17-21 December

2002

Polysialic acids in drug delivery
SMI
The Hatton, London, 13-14 February

Recent developments in liposome technology
Scientific Symposium on Vaccination against Influenza
Kemi, Finland, 21-24 March

Liposomal DNA CTL mediated responses in mice
WHO International Working Group on the Standardization and Control of Nucleic Acid Vaccines, seventh meeting
NIBSC, Potters Bar, UK, 21 June

Liposomes: A role in the delivery of DNA vaccines
AAPS Annual Meeting Short Course: Liposomes in Drug and Vaccine Delivery
Toronto, Ontario, Canada, 11-14 November

2003

Liposome-mediated DNA vaccines: Recent advances
IV Simpósio do NECF, Instituto Superior de Ciências da Saúde-Sul,

Lisbone, Portugal, 14 November

Co-delivery of plasmid DNA and the antigen it encodes via liposomes greatly enhances protective immunity: Application in the development of an influenza vaccine

6th International Conference Liposome Advances: Progress in Drug and Vaccine Delivery

School of Pharmacy, London, 15-19 December

Of the secondary documents cited by the Office, only Vaage and Saxon describe *in vivo* experiments with combinations of drugs. Since the problem to be solved by the invention is *how* to, translate the synergistic effects of combinations of drugs as ascertained *in vitro* to *in vivo* administration, the Bally document is essentially not relevant when the invention is considered in this light.[1]

Turning, then, to the two secondary references that do address *in vivo* administration, it is apparent that neither took any account of *in vitro* teachings that the non-antagonistic effect of drugs in combination is affected by its ratio. Vaage simply encapsulated vincristine and doxorubicin in separate vehicles for administration in apparently arbitrarily selected amounts and attempted to overcome the observed antagonism upon simultaneous administration by spacing the administration so that each drug was given alone at alternating 3 day intervals. No attempt was made to provide a *composition* that could be administered with at least additive effects. Saxon shows no evidence either of attempting to utilize any consideration of ratios or maintaining them in preparing the co-encapsulated drugs. In any event, the co-encapsulation composition fell apart.

Seen in this light, the Vaage and Saxon papers are themselves evidence that the invention compositions represent a departure from the thinking of those skilled in the art. If the compositions of the invention were obvious, neither Vaage nor Saxon would have done what they did - Vaage

[1] Nevertheless, the preparations of Bally as described in Part C in column 15 cannot be included within the scope of the compositions of the invention, not only because the ratio of drugs is antagonistic, as described in the Supplementary Amendment filed 9 December 2005, but also because the liposomes used are incapable of stable association with any drugs at all. The compositions of Bally are LUVs composed entirely of egg phosphatidylcholine (see line 39) and egg phosphatidylcholine vesicles are inherently incapable of stable association with drugs *in vivo*. This is verified by the enclosed publication of Scherphof, G., *et al.*, *Biochim et Biophys Acta* (1978) 542:296-307, entitled "Disintegration of Phosphatidylcholine Liposomes in Plasma as a Result of Interaction with High-Density Lipoproteins." In the summary, it is stated that "massive release of entrapped labeled albumin from the liposome during incubation with plasma suggests that the observed release of phosphatidylcholine from the liposomes has a highly destructive influence on liposomal structure." Thus, the compositions of Bally are of no help with respect to the present invention.

EXHIBIT 4

REMARKS

The disclosure of U.S. patent 5,736,155, submitted herewith in a formal Information Disclosure Statement, has come to the attention of the undersigned. Of particular relevance is column 15 under the heading "Part C" which describes liposomes loaded with either a combination of cytosine arabinoside along with doxorubicin or a combination of methotrexate along with doxorubicin.

One of the documents from which priority is claimed, U.S. provisional application 60/341,529 filed 17 December 2001, in working Example 3, prepares liposomes with combinations in the disclosed ratios and demonstrates that they fail to meet the limitation currently in claims 5 and 15, namely that a non-antagonistic effect is exhibited over at least 5% of the concentration range such that 80-20% of the cells are effected in an *in vitro* assay. For the convenience of the Examiner, the protocol of said Example 3 and the corresponding Figure showing results are included with this Supplementary Response as Exhibit A.

Accordingly, the limitations of claims 5 and 15 have been included in claim 1 and thus also in claim 13 from which they respectively depend. As the limitations inserted into claims 1 and 13 already were present in dependent claims, it is believed that no new issues are raised.

The undersigned apologizes for failing to have brought the attention of the Office to the '155 patent earlier.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 532552000100.

Respectfully submitted,

Dated: December 9, 2005 By: Kate H. Murashige

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synergistic interaction over a wide range of doses that affect 5% to 99% of cells (f_a =0.05 to f_a=0.99). In contrast, when the same agent combination is given at a different drug ratio, the interaction is strongly antagonistic over the same f_a range (Ratio 2 in Figure 2, cisplatin: topotecan 1:1).

Example 3

Combination effects of doxorubicin and cytosine arabinoside or doxorubicin and mitoxantrone

Doxorubicin: cytosine arabinoside (ratio of 1:0.45) and doxorubicin: methotrexate (ratio of 1:0.36) combinations were tested for additive, synergistic or antagonistic effects using the standard tetrazolium-based colorimetric MTT cytotoxicity assay protocol (Mosmann et al (1983) J Immunol Methods 65(1-2): 55-63) described above. Results from the MTT assay were used to calculate combination effects using the median-effect analysis described in the previous examples. The abovementioned ratios tested were based on ratios used in US patent no. 5736155, Bally et al. As depicted in Figure 3, the above indicated ratios displayed antagonistic combination effects over a substantial range of f_a values. It should be noted that data lying outside f_a ranges of about 0.2 to 0.8 are not reliable.

Example 4

Two agent combinations that exhibit synergistic combination effects

Combinations comprising vinorelbine, cisplatin, sphingosine and edelfosine in combination with sphingosine, edelfosine, camptothecin (topotecan), cisplatin and doxorubicin were tested for additive, synergistic or antagonistic effects using the standard tetrazolium-based colorimetric MTT cytotoxicity assay protocol (Mosmann et al (1983) J Immunol Methods 65(1-2): 55-63). Results from the MTT assay were used to calculate combination effects using the median-effect analysis described in the previous examples. Results are shown in Table III:

TABLE III

Doxorubicin in Combination with Cytosine Arabinoside or Methotrexate

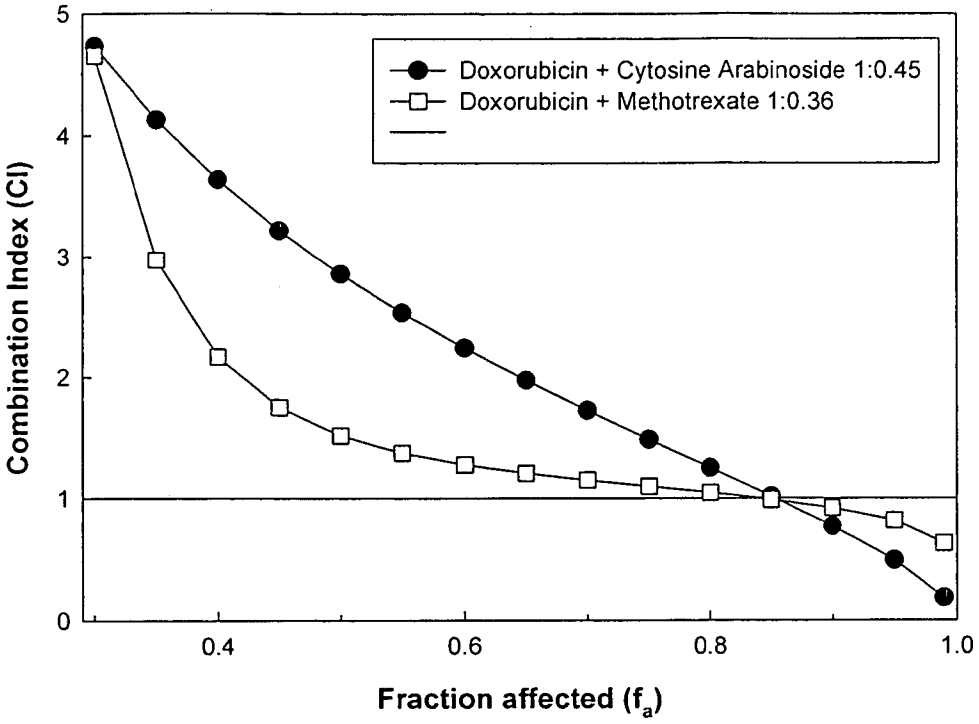


Figure 3

in the art and no attempt has been made in the art to obtain them. That is, there is nothing in the cited art that suggests that one even would want to obtain a composition where the administered drugs are maintained at a predetermined synergistic ratio in order to ensure delivery of a synergistic ratio at a tumor site in a clinical context. Since no one has suggested preparing a composition with these characteristics, the compositions cannot possibly be rendered obvious, they can only be inherently anticipated if, by some accident, compositions with the same characteristics were obtained. In order to defeat patentability, it is therefore necessary to provide a prior art document that describes a composition that has the same characteristics as that claimed, either explicitly or inherently. No such document has been found. In short, because no one in the art sought to obtain a composition with the required characteristics, such compositions cannot be obvious, they can only be coincidentally anticipated (which, in this case, they are not). This should be borne in mind in the context of the arguments below.

The Anticipation Rejection Over Vaage

Claims 1, 3, 6-7, 10-11, 13, 16-19, 46 and 48 were rejected as assertedly anticipated by Vaage. Applicants believe that the invention of claim 1 is distinct from Vaage thus distinguishing its dependent claims as well. The Examiner has properly raised the point that the burden is on applicants to show that the compositions taught by Vaage do not fall within the functional language claimed. Applicants are appreciative that the discussion at the interview resulted in an indication that this rejection may be withdrawn. A specific discussion of the points raised follows.

First, the composition in Vaage fails to meet the *in vitro* test specified in the claim. Enclosed herewith is a publication: Abraham, S. L, *Clin Cancer Res* (2004) 10:728-738, which shows that at all the vincristine:doxorubicin mole ratios tested against tumor cell lines (1:4, 1:7

EXHIBIT 6

and 1:20) were antagonistic. (See page 736 and figure 7.) Applicants have calculated the mole ratio of vincristine:doxorubicin in Vaage as 1:9.5. Thus, the ratio administered in the Vaage article is in the range reported antagonistic by Abraham.

And the drugs are antagonistic *in vivo* as well. The relevant data are shown in figure 5. This figure plots tumor volume against time. Doxil (liposomal doxorubicin) and S-VCR (liposomal vincristine) were simultaneously injected on days 3, 10 and 17 (Table V). The results for this combination are shown on line 6 as a dramatic increase in tumor volume exceeded only by placebo (line 1) and S-VCR (line 2). The result for the combination was even worse than that obtained when doxil was administered alone (line 3). Thus, the combination of liposomal vincristine and liposomal doxorubicin was clearly antagonistic.

In an attempt to overcome this, Vaage did not attempt to adjust the ratio of vincristine to doxorubicin, as is done in the present invention. Rather, Vaage teaches away from using a single composition by attempting (successfully) to overcome this antagonism by administering the drugs separately with a 3-day interval between administration of vincristine and administration of doxorubicin.

Thus, not only do the compositions of Vaage not meet the claim limitation of using non-antagonistic drug ratios, Vaage teaches away from even trying to use a single composition containing both drugs. This is evident from the data set forth above.

Applicants believe the foregoing meets their burden to demonstrate lack of anticipation, and are gratified that there is apparent agreement on this point. It is thus believed that the rejection for anticipation by Vaage can be withdrawn with respect to claim 1, and therefore with respect to all claims dependent thereon.

Docket No : 532552000100
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Paul TARDI et al.

Application No : 10/264,538 | Confirmation No : 5304

Filed: October 3, 2002 | Art Unit: 1615

For: COMPOSITIONS FOR DELIVERY OF DRUG COMBINATIONS | Examiner: G Kishore

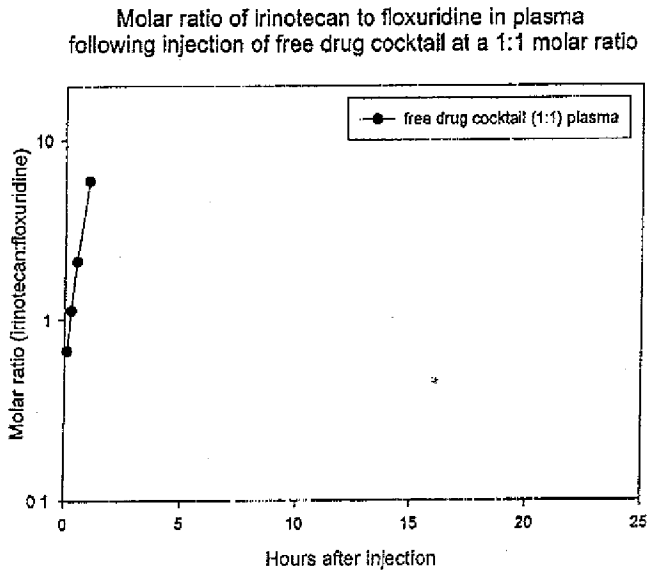
DECLARATION OF LAWRENCE D. MAYER

MS AF
Commissioner for Patents
P O Box 1450
Alexandria, VA 22313-1450

I, Lawrence D. Mayer, declare as follows:

1 I am President and Head of Research at Celator, the assignee herein. I conducted or supervised the conduct of the experiments described below.

2 In this study, CD-1 nude mice bearing Capan-1 tumors were administered IRI:FLOX in a 1:1 ratio as a free drug cocktail Blood ratios were measured as a function of time. The results are shown in the graph below.



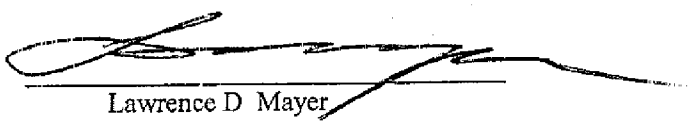
The original 1:1 ratio, which is non-antagonistic, becomes dramatically distorted almost immediately in the blood. After less than 1 hour, the IRI:FLOX ratio increased to 6:1, which is antagonistic.

3. These results clearly show that unless drugs are formulated so as to control their pharmacokinetics, because of their differences in metabolism, an initially administered ratio will not be maintained *in vivo* and can in fact invert from non-antagonistic to antagonistic.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by

fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at Vancouver, CANADA, on April 24, 2006.



Lawrence D Mayer